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Community ecology of insects inhabiting ephemeral habitats.

Ph.D. Thesis

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České Budějovice 2017

This thesis should be cited as:
Sládeček, FXJ, 2017: Community ecology of insects inhabiting ephemeral habitats. Ph.D. Thesis Series, No. 18. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 160 pp.

Annotation

The aim of this thesis was to investigate community assembly mechanisms driving the temporal patterns, succession and seasonality, in dung-inhabiting insects as a model community of insects inhabiting ephemeral habitats. I have shown that the succession of dung-inhabiting beetle and fly species follows the mechanisms of habitat filtering. This was reflected in species successional aggregation in adult dung-visiting flies, aggregation of beetle and fly functional groups in succession, sized-based successional patterns of dung-inhabiting beetle predators and, finally, by reflection of successional patterns of dung-emitted volatiles by beetle and fly species' succession. Seasonality follows the mechanisms of niche differentiation among adult flies and beetle predators, while it should rather follow principles of habitat filtering between all beetles and flies.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

České Budějovice, 15.9.2017

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František XJ Sládeček

This thesis originated from a partnership of Faculty of Science, University of South Bohemia, and Institute of Entomology, Biology Centre of the ASCR, supporting doctoral studies in the Entomology study programme.

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Financial support

The projects in this thesis were supported by Institute of Entomology, Biology Centre of the Czech Academy of Science (RVO//:60077344), by the Grant Agency of the University of South Bohemia (152/2016/P), Postdoc project of University of South Bohemia in České Budějovice (reg.no. CZ.1.07/2.3.00/30.0006), and by the Grant Agency of Czech Republic (15-24571S).

Acknowledgements

I am really grateful to everyone who contributed to the creation of this thesis, namely:

1) To God, since: [Psalms 23]

The LORD is my shepherd, I lack nothing.
He makes me lie down in green pastures,
he leads me beside quiet waters,
he refreshes my soul.
He guides me along the right paths
for his name's sake.
Even though I walk
through the darkest valley,
I will fear no evil,
for you are with me;
your rod and your staff,
they comfort me.

You prepare a table before me
in the presence of my enemies.
You anoint my head with oil;
my cup overflows.
Surely your goodness and love will follow me
all the days of my life,
and I will dwell in the house of the LORD
forever.

2) To my family, namely to my parents who supported me through undergraduate studies, and to my wife and daughter, who technically did not help, but at least made less obstacles for thesis' creation than they could.

3) To my supervisor, Martin Konvička, for giving me a free hand and financial support in whatever project I wanted to pursue, and who also had patient with my slower progress in certain parts of my PhD study.

4) To my colleagues, namely to (without particular order) Martin Volf, Simon Segar, Jan Hrček, Petr Klimeš, Lukáš Čížek, Robert Tropek and Aleš Bezděk for vital advises and support in both scientific work and real life.
5) To Tomáš Zítek, who both did not kill me during our three months-long trip to Africa, and who endured my first, clumsy, steps of being a supervisor.
6) To Stefan Dötterl and Irmi Schäffler from Salzburg for both eager start with our dung smells related co-operation (Chapter IV) and lasting patience with my slow progress due to me doing X other things simultaneously to our project.
7) To my friends/colleagues/students in Prague who still, hopefully, follow my bossing around.
8) To everyone, be it friend or a random innocent by-stander/sitter, who was willing to listen to my long talks about how studying dung ecology is the best thing since sliced bread.
9) Finally, I wanted to dedicate this thesis to memory of Ilkka Hanski (1953-2016), with whom I will never have a chance to co-operate (despite it was my dream). Since I do not know whether the thesis is worth his legacy, I am placing the dedication here, where probably no one will read it but at the same time I will still have the feeling of job well done....

Cover photo by Tomáš Zítek, 2015.

List of papers and author’s contribution

The thesis is based on the following papers (listed chronologically):

I. Frantisek Xaver Jiri Sladecek, Hana Sulakova, Martin Konvicka. Temporal segregations in the surface community of an ephemeral habitat: Time separates the potential competitors of coprophilous Diptera. Entomological Science 20 (2017): 111-121. doi: 10.1111/ens.12240. (IF = 1.262 (2016)). *FXJS sampled the data, formulated hypotheses, performed the statistical analyses, wrote first draft of the manuscript.*

II. Frantisek Xaver Jiri Sladecek, Simon Tristram Segar, Colin Lee, Richard Wall, Martin Konvicka, 2017. Temporal segregation between dung-inhabiting beetle and fly species. PLoS ONE 12(1): e0170426. doi: 10.1371/journal.pone.0170426. (IF = 2.806 (2016)). *FXJS sampled the Czech datasets, formulated hypotheses, performed the statistical analyses, wrote first draft of the manuscript.*

III. Frantisek Xaver Jiri Sladecek, Tomas Zitek, Martin Konvicka, Simon Tristram Segar. How do the temporal trends of dung-inhabiting predators affect their coexistence? (manuscript). *FXJS sampled the data, formulated hypotheses, performed the statistical analyses, wrote first draft of the manuscript.*

IV. Frantisek Xaver Jiri Sladecek, Stefan Dötterl, Irmgard Schäffler, Simon Tristram Segar, Martin Konvicka. Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds. (manuscript). *FXJS sampled the insect and chemical data, participated in processing of chemical data, formulated hypotheses, performed the statistical analyses, wrote first draft of the manuscript.*

Co-author agreement:

Martin Konvička, the supervisor of Ph.D. thesis and co-author of all presented papers and manuscripts, fully acknowledges the major contribution of František XJ Sládeček in all presented papers.

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Doc. Mgr. Martin Konvička, Ph.D

Simon T. Segar, the co-author of paper II and manuscripts III and IV, acknowledges the major contribution of František XJ Sládeček in those presented paper and manuscripts.

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Simon T. Segar, Ph.D.

Contents

INTRODUCTION

What are ephemeral habitats and why we should study them?

Ephemeral habitats such as dung pats, animal carrion, rotten fruit and fruiting bodies of Macromycetes are spatially well define yet temporally unstable patches (Finn 2001). During their short existence they, however, provide a high energy and nutrient content for their associated communities, e.g. nitrogen in dung (Gittings & Giller 1998; Holter & Scholtz 2007). Ephemeral habitats are therefore inhabited by a wide array of animal, fungal and bacterial species (Masunga *et al.* 2006; Lukasik & Johnson 2007; Yamashita & Hijii 2007; Pechal *et al.* 2013; Sladecek *et al.* 2013).

Owing to the diversity of their communities and their temporally and spatially limited nature (Finn 2001), ephemeral habitats could provide a solid model for studies of their communities' coexistence, yet their potential has not been fully utilized. The vast majority of ecological studies has historically focused on communities of plants (Clements 1916; Gleason 1926; Connell & Slatyer 1977; Keddy 1992; Silvertown 2004; Kraft *et al.* 2015), including other biota closely following the plant dynamics (Macarthur 1958; Schoener 1974; Novotny *et al.* 2006; Fowler, Lessard & Sanders 2014). The only exception were studies focusing on benthic sessile animal communities (Farrell 1991; Benedetti-Cecchi 2000; Maggi *et al.* 2011). Sessile animals, however, share so many traits with plant communities, that we could consider them as "plant-like" communities. In both plants and sessile animal communities, temporal development proceeds, mostly slowly, from virtually no community presented to increasingly complex and more or less stable community (Cook 1996; Maggi *et al.* 2011). In contrast, the temporal development in ephemeral habitats proceeds, mostly rapidly, from the highest amount of resources to no resources in the end (Gittings & Giller 1998; Kocarek 2003; Lee & Wall 2006; Lukasik & Johnson 2007). The community development varies among ephemeral habitats with highest diversity and abundance at the start in dung (Mohr 1943; Koskela & Hanski 1977; Sladecek *et al.* 2013), in the middle of development in carrion (Tabor, Fell & Brewster 2005; Sharanowski, Walker & Anderson 2008; Matuszewski *et al.* 2011) or at the end of ephemeral habitat's existence in rotting fruit and fungi (Lukasik & Johnson 2007; Yamashita & Hijii 2007). In addition to high diversity and abundances of their communities over limited period of time, and thus a great potential for species coexistence studies, ephemeral habitats can provide a logistically very sustainable models for ecological studies. In contrast to plants and their associated communities which usually develop over decades, community dynamics can be fully resolved in matter of days (dung, fruit falls) (Lukasik & Johnson 2007; Chao, Simon-Freeman & Grether 2013; Sladecek *et al.* 2013) to maximum of several months in very large carrion (Tabor, Fell & Brewster 2005; Sharanowski, Walker & Anderson 2008; Matuszewski *et al.* 2011).

Apart from being logistically and ecologically interesting model for ecological studies, communities inhabiting ephemeral habitats also provide great services to other natural communities, and also to humanity itself. The primary role of such communities is destruction of their habitats and recycling of nutrients for mostly the plant communities. Such service is especially prominent in dung-inhabiting communities whose species pull dung portions into ground, aerate the soil or just destroy the pats by their activity (Edwards & Aschenborn 1987; Stevenson & Dindal 1987; Slade *et al.* 2007; Wu, Griffin & Sun 2014; Tixier, Lumaret & Sullivan 2015). Without those communities, animal husbandry would be impossible as dung would soon cover most of the pastures. Such risk even lead to introduction of more "efficient" dung-inhabiting beetles to Australia and Texas where indigenous fauna was not able to process the dung mass excreted by introduced cattle (Bornemissza 1979). While carrion-inhabiting communities provide practically the same environmental service, their community development also serves humanity for the purpose of legal investigation (forensic entomology) with earliest mentions dating back to the 13th century (Benecke 2001; Amendt *et al.* 2011). Finally, communities inhabiting ephemeral habitats provide a service of "self-policing" some of their negative elements for humanity. This includes development suppression of economically important pest dung flies (Ridsdillsmith, Hayles & Palmer 1986; Roth, Macqueen & Bay 1988), internal parasites distributed by dung (Nichols & Gomez 2014) or medically important carrion-inhabiting bacteria (Mumcuoglu *et al.* 2001).

Inhabitants and general ecological patterns of ephemeral habitats

Ephemeral habitats are inhabited by a wide array of species from animals, fungi to bacteria. The most attention was given to the animal species, primarily to their definite effect on destruction of the ephemeral habitats, e.g. dung relocating beetles (Slade *et al.* 2007) or vertebrate scavengers on carrion (Allen *et al.* 2014), secondly to less methodological issues when studying them.

Among animals, invertebrates form a core of communities inhabiting all ephemeral habitats. Despite vertebrates do play a significant role in carrion, they, however, can both destroy carcasses or facilitate invertebrates' activity (Allen *et al.* 2014). Among invertebrates, insect play the most crucial role in destruction of ephemeral habitats, either by destroying them itself (Suzuki 2000; Tixier, Lumaret & Sullivan 2015), by facilitation of other invertebrates' activity, e.g. earthworms in dung (Holter 1977), or facilitation of fungal and bacterial colonization and activity (Lussenhop, Kumar & Lloyd 1986; Stevenson & Dindal 1987; Blackwell & Malloch 1991; Greif & Currah 2007). In dung, carrion and rotting fungi, beetles and Diptera form the main insect groups (Mohr 1943; Yamashita & Hijii 2007; Matuszewski *et al.* 2010). The role of other insect groups is usually restricted to either single type of ephemeral habitats, e.g. butterflies in rotten fruit (Lukasik & Johnson 2007), ants in carrion (Lindgren *et al.* 2011), or to specific conditions, e.g. termites decomposition of dung in parts of year when beetles and Diptera are not active (Coe 1977). In this thesis, I will therefore focus primarily on ecology of beetle and dipteran species that inhabit ephemeral habitats.

Beetle and dipteran species inhabiting ephemeral habitats can be classified into three functional groups. The first are the SAPROPHAGES (i.e. coprophages, necrophages, etc.). Even though some developmental stages can also consume living matter, e.g. larvae of some dung beetles (Landin 1961) or some larval instars of dung and carrion inhabiting Diptera (Skidmore 1985; Rosa *et al.* 2006), such species should primarily consume the decaying matter (Koskela & Hanski 1977). In general, some form of specialized morphology is presented in those species, e.g. mouthparts for filtrating only small nitrogen-rich particles from dung in adult dung beetles (Scarabaeidae) (Holter 2000; Holter, Scholtz & Wardhaugh 2002; Holter & Scholtz 2005) and dipteran larvae (Dowding 1967). The second group are the OMNIVORES. Such species change their feeding mode from saprophagy to predation, or vice versa, between adult and larval stage (Koskela & Hanski 1977; Sowig 1997). The prime example are dung-inhabiting Hydrophilidae species who are specialized saprophages as adults, having the filtrating mouthparts (Holter 2004), and predatory as larvae (Sowig 1997). The opposite development direction is represented by carrion-inhabiting *Nicrophorus* species (Silphidae), who prey upon carrion-inhabiting dipteran larvae as adults but their larvae are specialized saprophages (Scott 1998). The third group are the PREDATORS. Those species are predatory in both adult and larval stages (Koskela & Hanski 1977). This group also include parasitoids, both beetles and Hymenoptera (Greene 1997; Horenstein & Salvo 2012).

Parental care, nest constructions and, most importantly, relocation of resources from original habitats are the most peculiar ecological traits in insects inhabiting ephemeral habitats. Despite this behavior is limited primarily to dung and carrion communities (Halffter & Edmonds 1982; Scott 1998), with some potential in rotten fungi (Frolov, Akhmetova & Scholtz 2008), the role of resource relocation could have a tremendous effect on development of such habitats. Predominantly because resource relocating species can sometimes easily deplete the original habitats' mass (Suzuki 2000; Horgan & Fuentes 2005). Therefore for ecological interpretations, the functional groups of saprophages and omnivores must be further split into the guilds of RELOCATORS, species that relocate their resource, and DWELLERS, species that do not relocate their resource and whose larvae live in original habitats (Davis 1989; Gittings & Giller 1997). Relocators themselves could be further split into horizontal relocators, species that form a spherical (ball) object from the material and roll it away from the source (dung beetles; rollers), or to vertical relocators who bury the resource under the original source (dung beetles, *Nicrophorus* species; tunellers) (Doube 1991; Scott 1998).

The most apparent and the most studied ecological processes involving ephemeral habitats are the succession and seasonality of their species. Over the last century, an abundance of studies documenting those temporal trends has appeared, focusing either on ecological interpretations of species coexistence (dung, rotten fungi and fruits, carrion to some extend) (Hammer 1941; Mohr 1943; Kocarek 2003; Yamashita & Hijii 2007; Sladecek *et al.* 2013; Mroczunski & Komosinski 2014; Pechal *et al.* 2014), or for purpose of legal investigations (carrion) (Castro *et al.* 2012; Castro *et al.* 2013; Matuszewski, Szafalowicz & Grzywacz 2014). Both successional and seasonal gradients of species segregations have been widely considered to play, probably the most, important role in coexistence of such communities, primary by reduction of potential competition (Hanski & Koskela 1979; Guevara *et al.* 2000; Kocarek 2003; Sladecek *et al.* 2013). In this thesis, I will therefore further try to exanimate whether succession and seasonality are really facilitating the coexistence of communities inhabiting the ephemeral habitats by providing niche separations among constituent species, or are results of species adapting to environmental conditions.

Coexistence of communities inhabiting ephemeral habitats

General mechanisms of species coexistence

Mechanisms of species coexistence in natural communities are the key issue in community ecology, since without them there would be no communities but single species dominated assemblages (Gause 1934). Two main mechanisms are usually considered in maintaining the diversity in natural communities in contemporary science.

The first one is based upon species shifts in their resource utilization, the niche differentiation, leading to decrease or even elimination of negative interactions between species (MacArthur & Levins 1967; Schoener 1974; Silvertown 2004). These negative interactions could be either indirect or direct. Indirect interactions include competition via depleting each others' resource (exploitative competition) (White, Wilson & Clarke 2006), be it nutrient in plants and primary consumers (Vanderhaeghe *et al.* 2016) or their prey species in predators (White, Wilson & Clarke 2006), or by increasing the pressure on their competitor via increasing abundance of their mutual enemy, primarily the predator (apparent competition) (Holt & Lawton 1993). Direct interaction include mortality or serious harm induced between competing species, be it just harming or killing the competitor (interference competition) (Hawes, Evans & Stewart 2013) or predation among predators (intra-guild predation) (Holt & Huxel 2007; Gagnon, Heimpel & Brodeur 2011; Raso *et al.* 2014).

Niche differentiation predicts coexistence of species with different traits defining their niches (Maire *et al.* 2012), primarily along temporal, spatial and resource selection axes (Schoener 1974). Temporal segregations involve using the same resource but in more or less differing time periods (Crumrine 2005; Adams & Thibault 2006; Bischof *et al.* 2014; de Camargo *et al.* 2016). Spatial segregations include again using the similar resource but avoiding competition by separation from slight vertical stratification (Opatovsky *et al.* 2016) to larger scale range separations (Droge *et al.* 2017). Finally the resource shifts include specialization to utilization of different resource among co-occurring potential competitors, e.g. host-specificity in parasitoids of herbivorous insects (e.g. Van Veen *et al.* 2008; Hrcek *et al.* 2013).

The second principle of species coexistence is based upon general adversity of environment conditions to species fitness, the habitat filtering (Keddy 1992; Kraft *et al.* 2015). Such environmental adversities range from various abiotic conditions such as temperature (Nisimura, Kon & Numata 2002; Verdu *et al.* 2007; Verdu, Alba-Tercedor & Jimenez-Manrique 2012), humidity (Ramos, Diniz & Valls 2014) or resource availability to conditions induced by their host species, such as plant produced chemical for herbivorous species (Volf *et al.* 2015), or their prey defenses for predatory species (Kajita *et al.* 2014). Habitat filtering thus predicts coexistence of species with similar traits related to overcoming such environmental challenges (Maire *et al.* 2012).

Niche-based coexistence of species inhabiting ephemeral habitats

Niche based species segregation, especially succession and seasonality, has always been suggested as a main mechanisms supporting coexistence of communities inhabiting dung (Sladecek *et al.* 2013) with few suggestions from rotten fruit-feeding (Lukasik & Johnson 2007) and fungal-dwelling communities (Guevara *et al.* 2000). The role of succession in niche segregation is, however, overestimated in compare to seasonality or spatial segregation.

The necessary condition for exploitative competition is that the community contains species able to monopolize the ephemeral habitat. The indirect exploitative competition via depleting the resource for saprophagous species is well documented phenomenon in dung (Giller & Doube 1989), carrion (Suzuki 2000) and to less extend in rotten fruit (Lukasik & Johnson 2007). In dung, such competition occurs in communities with significant representation of large dung relocating beetles (Krell *et al.* 2003). This is especially prominent in tropics, where ball-forming and large quickly burying relocators, who immediately bury dung needed for nest construction, can deplete even large piles of dung in a matter of minutes-hours (Hanski & Cambefort 1991). Slow burying relocators, who bury dung and construct nests continuously, are therefore forced to temporally displacements by activity of their competitively dominant kin, usually forming a wide array of temporal optima outside the main activity of dominant species (Edwards & Aschenborn 1987; Krell-Westerwalbesloh, Krell & Linsenmair 2004). Dwellers, who construct nests within the dung pats, are often forced to either complete temporal segregation from dominant species, thus occurring in different part of season (Davis 1989), or to spatial segregations, utilizing dung dropped in shades or on soils that hamper the relocating ability of dominant species (Davis 1994; Giller & Doube 1994; Krell *et al.* 2003). In both temperate and tropical carrion communities, relocating species can quickly monopolize smaller carcasses (Suzuki 2000), thus forcing the rest of species to temporal segregations via seasonality or to use larger carcasses (Anderson 1982), which in turn could be more prone to destruction by vertebrate scavengers (Allen *et al.* 2014). The rotten fruit could be easily monopolized by foraging termites (Lukasik & Johnson 2007).

In contrast, exploitative competition is probably of small or no importance in communities without such dominant species. In dung-inhabiting temperate communities, only a small portion of initial energy presented in dung is utilized by adult coprophagous beetles (Holter 1975). Although some exploitative competition was suggested for beetle saprophagous larvae (Landin 1961), no further studies were carried out (Finn & Gittings 2003). However, even though such larval competition took place, coprophagous beetles could avoid it via their adults' fine-scale seasonal segregation (Hanski 1986; Gittings & Giller 1997; Sladecek *et al.* 2013), as their larvae co-occur in succession (Landin 1961). Another problem when evaluating exploitative competition in dung is a lack of quantitative data regarding the ecological patterns of dung-inhabiting

Diptera, both adults and larvae (Finn & Gittings 2003). Such larvae could be in exploitative competition with beetles, via beetles' dung pat shredding activity (Hanski & Cambefort 1991). Resolution to such competition could again lie in seasonality as dipteran species seem to be most abundant in summer avoiding the beetle spring and autumns abundance peaks (Hammer 1941; Hanski 1986; Gittings & Giller 1997; Sladecek *et al.* 2013). In carrion, necrophagous dwellers are known to avoid carcasses with high abundance of blowfly larvae (Blackith & Blackith 1990), but this is probably more due to their interference competition.

Contrary to exploitative competition in saprophages, very little is known about exploitative competition among predators, who inhabit the ephemeral habitats. The exploitative competition between dung-inhabiting predators is considered to be of a small importance due to sheer abundance of their potential prey (Valiela 1974), even though some predators were to be able to process considerable amounts of prey (Valiela 1969). Finally, virtually nothing is known about apparent competition in ephemeral habitats, although there could be some potential for it in carrion, as rate of parasitism does seems to increase sharply with abundance of dipteran larvae in season (Horenstein & Salvo 2012).

The direct interactions, interference competition and intra-guild predation, in ephemeral habitats are, again, very understudied. Among saprophages, larvae of temperate coprophagous beetles could involve in interference competition, by killing or even partly eating the opponent (Landin 1961). As with exploitative competition, this negative interaction could be resolved by species seasonality. In carrion, larvae of flesh flies are known to kill co-occurring larvae of blowflies (Blackith & Blackith 1990), who, contrary to them, occur in very large quantities (Matuszewski *et al.* 2010). Larvae of blowflies are in turn known to produce ammonia based antimicrobial substances that also probably deter necrophagous dweller beetles (Lennox 1940; Blackith & Blackith 1990). Some of those beetles thus avoid this interference by seasonal shifts to months when larvae of blowflies are less abundant (Kocarek 2002). Practically nothing is known about interference competition and intra-guild predation among predators inhabiting ephemeral habitats. The only information we posses is that increase in dung-inhabiting predators' abundance past certain predator:prey ratio does not increase overall predation (Roth 1982; Fincher 1995), which strongly suggests either interference competition or intra-guild predation.

Environmental filtering in ephemeral habitats

As ephemeral habitats are from their definition ever changing in time, their communities are faced with changing physical and chemical properties, which usually develop from very unfriendly in the very fresh ephemeral habitats, e.g. high moisture of dung (Lysyk, Easton & Evenson 1985; Gittings & Giller 1998), to more favorable when the habitat is almost processed by its community. In addition, as every organism on Earth, communities inhabiting ephemeral habitats must adopt to survive the ambient conditions around their ephemeral habitats. In contrast to niche differentiation, both succession and seasonality of species inhabiting ephemeral habitats seem to follow the rules of habitat filtering.

Ambient temperature is probably the main environmental variable affecting the communities inhabiting ephemeral habitats outside of those habitats. In general, high ambient temperature is harmful to beetles (Landin 1961; Nisimura, Kon & Numata 2002; Merrick & Smith 2004) and beneficial for dipteran species, who need higher temperatures for commencing their activity (Hammer 1941; Matuszewski, Szafalowicz & Grzywacz 2014) in both dung and carrion. Therefore beetles should occur primarily in cooler parts of a year (Kocarek 2003; Sladecek *et al.* 2013), while dipteran species should occur primarily in hotter parts of the year (Hammer 1941). Among dung-inhabiting beetles, only ball-forming relocators have some affinity for higher temperatures (Krell-Westerwalbesloh, Krell & Linsenmair 2004). This affinity is, however, tied to various heat reduction adaptations (Verdu, Diaz & Galante 2004; Verdu, Alba-Tercedor & Jimenez-Manrique 2012). Dung-inhabiting temperate dwellers occurring throughout the summer also have do posses some form of heat resistance, as they can survive higher temperatures than their spring/autumn counterparts, but even they may perish under higher than average summer temperatures (Landin 1961). High temperatures also prevent carrion burying beetles from nest constructions (Nisimura, Kon & Numata 2002).

Physical properties of ephemeral habits could be characterized as rather adverse to associated animal communities. Dung moisture content is the prime example. The moist parts of dung pats could be generally lethal to beetles (Whipple, Cavallaro & Hoback 2013). In addition, only fraction of beetle community was found to inhabit the wettest portion of dung pats (Holter 1982). In carrion, the adversity is the fresh skin and lack of open body cavities that prevent insect colonization (Pechal *et al.* 2014). In rotten fruit, the same could apply to fruit peels (Lukasik & Johnson 2007). All such physical barriers gradually soften throughout ephemeral habitats' ageing and succession of their communities. In both dung and carrion, the community composition develops from habitat specialists to habitat generalists (Hanski & Koskela 1977; Koskela & Hanski 1977; Sharanowski, Walker & Anderson 2008). Similarly in both habitats, activity of early successional species should facilitate the activity of late successional species (Lumaret & Kadiri 1995; Lee & Wall 2006; Pechal *et al.* 2014). Finally, ability to relocate dung in beetles, who are generally the very early successional species (Krell-Westerwalbesloh, Krell & Linsenmair 2004; Sladecek *et al.* 2013), is considered as potential mean of dealing with high initial dung moisture (Halffter & Edmonds 1982; Gittings & Giller 1998).

Contrary to physical characteristics, chemical properties of ephemeral habitats are generally more positive towards their communities. Primarily, insects detect the ephemeral habitats using the chemical volatile compounds released from dung, carrion, rotten fungi and fruit (Dormont *et al.* 2007; Drilling & Dettner 2009; Segura *et al.* 2012; von Hoermann *et al.* 2013; Midgley *et al.* 2015). Unfortunately, the majority of studies just end with such information. Volatile compounds released along gradient of habitat ageing were studied only in carrion, however, for the purpose of legal investigations (Dekeirsschieter *et al.* 2009; Forbes & Perrault 2014; Paczkowski *et al.* 2015; Perrault *et al.* 2015). Therefore, no attempt was made to interpret the colonization patterns of carrion-inhabiting insects in light of those chemical volatiles. In dung, the major focus was just given to beetles' dung type selection from array of domesticated ruminants. Some fine scale preferences were found (Dormont, Epinat & Lumaret 2004; Dormont *et al.* 2007; Dormont *et al.* 2010). Among negative known impacts of ephemeral habitats' chemistry, the insects inhibiting rotting fungi are initially inhibited by fungi chemistry, similarly to dung moisture, and colonize the fungus when this adverse chemicals are gone (Jonsell & Nordlander 2004; Orledge & Reynolds 2005).

Aims and Scopes

Communities inhabiting ephemeral habitats are studied relatively frequently as is evident from previous sections. However, the scope of the studies in individual habitats slightly differ. While studies of dung, and to some extend rotting fungi are mostly focused for purpose of ecological interpretations (Yamashita & Hijii 2007; Sladecek *et al.* 2013), studies of carrion are predominantly focused on establishing just the species successional patterns for purpose of forensic entomology (Matuszewski *et al.* 2010; Matuszewski *et al.* 2011), and rotting fruit is rather understudied (Lukasik & Johnson 2007). I will therefore focus solely on communities inhabiting animal dung in this thesis, as dung was studied in more ecological way and there are also more studies focusing on dung-inhabiting communities than there are on rotting fungi. Despite the abundance of studies focusing on dung-inhabiting communities, the vas majority focused just on beetles, and mostly just on beetle saprophages.

The very aim of this thesis is therefore to explore several topics that are usually overlooked in studies of dung-inhabiting communities. The another problem of studies focusing on ephemeral habitats is general segregation from studies of other natural communities, e.g. dung and plants, and even from other studies involving ephemeral habitats, e.g. dung and carrion, when discussing the results. My secondary goal in this thesis is therefore an attempt to both include interpretations based upon results from all ephemeral habitats as well as from studies of other natural communities. By doing so, I incorporate dung community into larger scale of ecological framework.

Chapter I of this thesis focuses on ecology of dung-visiting dipteran species, as until present, there are very few studies quantitatively exploring their ecology. I studied succession and seasonality of adults dipteran species that perch on the top of dung pats to investigate how those trends could contribute to their coexistence. I have shown that despite there is some successional separation, the very early and very late successional groups are seasonally separated from the mid successional groups. In addition, species within the bulk of the mid successional group are usually separated via their more finely defined seasonal optima.

Chapter II investigates temporal patterns, succession and seasonality among functional groups of dung-inhabiting beetles and flies, including both adults and larvae. In succession, functional groups of both beetles and flies highly overlapped, suggesting the habitat filtering dynamics of succession in animal dung. In contrast, in season functional groups of beetles and Diptera displayed always some pattern of avoidance, which was perfectly reflected in patterns of individual species. While discussing both niche and environmental background of such patterns, habitat filtering via different beetle and Diptera temperature tolerances seems to more parsimonious explanation for such patterns.

Chapter III of this thesis investigates the potential competitive and intra-guild predation of dung-inhabiting predators, asking how their succession and seasonality promote their coexistence via lowering or elimination of their negative interactions. I have shown that the succession of dung-inhabiting predators follows a sized-based pattern in adult beetles, from largest to smallest, supporting the view that habitat filtering drives the dynamics of dung-inhabiting predators' succession. In contrast, species of varies sizes co-occur along seasonal gradient, which strongly indicates a role of niche differentiation among otherwise successionally co-occurring species. The combination of both the succession and seasonality extremely reduces or even eliminates potential competitive and intra-guild relations among dung-inhabiting predators.

In Chapter IV of this thesis, investigates the successional dynamics of dung emitted volatile compounds. I have then correlated the amounts of such volatiles with dung-inhabiting beetle and Diptera abundances, species richness and successional trends of individual species. I have shown that there is a succession of volatiles along the gradient of dung pats' ageing, but this succession rather consist of two successional groups rather than fluid succession of individual compounds. Only positive correlation occurred between the number of dung emitted volatile compounds and dipterans' abundance and species richness. The individual species of beetles and dipterans were, however, predominantly associated via their successional optima with either early successional volatile compounds (Diptera) or late successional volatile compounds (beetles).

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CHAPTER I

Frantisek Xaver Jiri Sladecek, Hana Sulakova, Martin Konvicka. *Temporal segregations in the surface community of an ephemeral habitat: Time separates the potential competitors of coprophilous Diptera.* Entomological Science 20 (2017): 111-121.

Entomological Science (2017) **20**, 111–121 doi: 10.1111/ens.12240

ORIGINAL ARTICLE

Temporal segregations in the surface community of an ephemeral habitat: Time separates the potential competitors of coprophilous Diptera

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Abstract

Temporal separations among species greatly enhance the species' coexistence, especially in insect communities inhabiting temporally unstable, yet resource-rich, ephemeral habitats like dung or carrion. The insect communities inhabiting ephemeral habitats consist of two components, the internal community dwelling within the substrate (mostly Coleoptera), and the surface community inhabiting the habitat's outer rim (mostly adult Diptera). In contrast to the internal community, the surface community is very rarely studied. We present here the first quantitative study of temporal trends in the surface community of coprophilous dipteran adults. Using artificially created 1.5 L cow dung pats, we studied the succession and seasonality in the surface community during six sampling periods in 2011 and 2012. In total, we sampled 13579 adults of dung-visiting Diptera. Both the abundance and species richness decreased rapidly throughout the succession, and were highest during summer. Along the successional gradient, the community was separated into two main groups (early and late) and four subgroups: (i) species occurring during the first few hours (mainly Calyptratae: Diptera); (ii) species occurring between the first and second days; (iii) species occurring between the second and third days (mainly Acalyptratae: Diptera); and (iv) species with optima after the third day of dung pat existence (mainly Nematocera). The earliest and latest successional groups, occurring mainly during spring–autumn, were seasonally separated from the two mid-successional groups, occurring during summer. The ecologically similar species displayed detectable seasonal micro-optima, which likely facilitate their coexistence. There was a high overall similarity in temporal patterns between dung and carrion surface communities.

Key words: dung flies, Muscidae, seasonality, Sepsidae, Sphaeroceridae, succession.

INTRODUCTION

The temporal aspects of natural communities, succession and seasonality, both facilitate the coexistence of ecologically similar species (Shimadzu *et al.* 2013). They are therefore favorite research subjects in community ecology. Despite this popularity, the understanding of temporal aspects of species coexistence remains incomplete for many taxa and communities.

Communities inhabiting ephemeral habitats are popular model communities to study the effects of temporal segregations. Ephemeral habitats are characterized by high nutritional content, discontinuous and unpredictable spatial occurrence and, most importantly, temporal instability (Finn 2001). Owing to this instability, ephemeral habitats provide a great and easily replicable model system for studies of temporal trends. The succession lasts for days or weeks (rarely months) there, compared to years in more stable communities of plants and sessile animals (Walker *et al.* 2010; Maggi *et al.* 2011). Examples of such systems include animal droppings (Lee & Wall 2006), carcasses (Sharanowski *et al.* 2008), rotten fruit (Lukasik & Johnson 2007) and fruiting bodies of Macromyceta

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Received 23 March 2016; accepted 29 August 2016; first published 16 November 2016.

fungi (Yamashita & Hijii 2007). Among those habitats, the temporal segregations have been most extensively studied in carrion and dung pats, both in European and North American temperate regions (e.g. Wingo *et al.* 1974; Hanski & Koskela 1977). In this study, we focused predominantly on dung-inhabiting (coprophilous) communities, using the carrion communities as a reference for comparison. We specifically focus on the insect community inhabiting cattle dung, since studies from other dung types, like horse dung (Psarev 2002; Mroczunski & Komosinski 2014), are scarce.

Successional and seasonal changes at both dung and carrion microhabitats can be observed independently among two main taxonomical and spatial compartments: (i) the surface community inhabiting the surface or outer rim of the habitat patch and containing mainly adult Diptera; and (ii) the internal community inhabiting the habitat patch interior and containing mainly coleopteran adults and dipteran larvae. This division was first used by Mohr (1943) to separate adult Diptera from adult Coleoptera, which both occur only sparsely in each other's dung dimension.

The temporal segregation within the internal community of coprophilous and necrophilous insects has been extensively studied (e.g. Hanski & Koskela 1977; Matuszewski *et al.* 2011) in quantitative (coprophilous) and semi-quantitative (necrophilous) manners. In both habitats, the saprophagous (copro- or necrophagous) species are separated along the successional gradient by their oviposition behavior: predators are distributed evenly and omnivores are usually early successional (Kocarek 2003; Sladecek *et al.* 2013). At the seasonal scale, the main separation, in both communities, is again between saprophages and omnivores, which avoid each other numerically in season (Benbow *et al.* 2013; Sladecek *et al.* 2013).

The temporal patterns in dung surface communities have been studied in a rather qualitative manner (Koskela & Hanski 1977). The most important and relevant studies were carried out by Hammer (1941) and Mohr (1943), both on cow-dung surface communities. Both authors reported the successional turnover between early successional Calyptratae Diptera and late successional Acalyptratae: Diptera. The same seems to apply also for adult Diptera on carrion (Grassberger & Frank 2004; Tabor *et al.* 2004; Bonacci *et al.* 2011). Both former authors also reported that early successional coprophilous fly species colonize the dung for only a very short time (in a matter of minutes; Hammer 1941; Mohr 1943). At the seasonal scale, there was a separation between the two most abundant Acalyptratae groups, with Sepsidae occurring mainly in summer and Sphaeroceridae occurring in spring and autumn (Hammer 1941; Mohr 1943; Laurence 1954).

Still, there are basically no quantitative studies of temporal trends in ephemeral habitat surface communities, as compared to the abundance of studies targeting the internal communities. The dung surface community quantitative studies by Papp (1992) were mostly limited to accumulations of individual species across habitats. The carrion surface community was studied more extensively for the purposes of forensic entomology (e.g. Grassberger & Frank 2004; Matuszewski *et al.* 2011); however, available data are semi-quantitative or qualitative (Michaud *et al.* 2012).

Because the ephemeral dung surface communities did not receive enough attention, we present here a first quantitative study of temporal segregations in a surface community of adult coprophilous Diptera. Our aim was to detect the roles of succession and seasonality in structuring this community. We focus our interpretations on both the taxonomic affiliations and ecological roles of individual species. We specifically focused on: (i) the temporal patterns of the whole community, i.e. changes in abundance and species richness; and (ii) temporal patterns among individual species, with regard to both the taxonomy (in family rank) and ecological affiliations (the guild membership).

MATERIALS AND METHODS

Study site

The study was carried out on a 23 ha pasture 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 vegetation season from March to October (Sladecek *et al.* 2013). The study site has a typical temperate continental climate with a very warm summer (average maximum temperature of 26°C and minimum temperature of 13°C in July) and cold winter (average maximum temperature of 3°C and minimum temperature of −4°C in January).

Diptera sampling

In total six sampling periods were carried out: three in 2011 (early spring, 23 April – 1 May; peak summer, 16–24 July; and late summer – early autumn, 26 August – 3 September) and three in 2012 (late spring, 9–17 May; peak summer, 27 July – 4 August; and late autumn, 14–22 September).

Dipteran communities were sampled from artificially created 1.5 L dung pats created from freshly defecated dung of permanently stalled cows. On the first day of each sampling period, five dung pats, situated 10 m apart and with 5 min time delay, were exposed between 11:00 h and 12:00 h. Each of those five pats represented one replication during the sampling period. The whole sampling period was planned to take place in a week with no major significant rainfall predicted to protect the sampling from potential weather influences. Each sampling period started on a bright, warm, sunny day to ensure that the dipteran adults were fully active.

From each of those pats, the dipteran communities were sampled 1, 2, 3, 5 and 7 h after placing the dung pat on the first day of dung pat existence. On the second day, the dung pat was sampled at 24, 27 and 31 h existence (11:00 h, 14:00 h and 18:00 h). On further days, the dung pat was sampled only at 11:00 h, and thus samples of the dipteran communities present on the dung pat of age 48, 72, 96, 120, 144, 168 and 192 h (2, 3, 4, 5, 6, 7 and 8 days) were obtained.

This repeated sampling approach was based both on Hammer's (1941) observation that dipteran adults chased away from dung by cattle or other disturbances return almost immediately to the same or a very similar composition. The dung pats were created at 12:00 h, since most species show their optima of daily activity around 12:00 h or in the early afternoon (Hammer 1941).

Individual samples were obtained by rapidly covering the pat with a sweeping net. The net bag was held conically above the pat, and the flies were disturbed and forced to fly upward into the bag by gently patting the dung while shaking the net.

The dipteran sample was then removed from the net and individuals were killed in a sampling bottle and taken to a laboratory for sorting and species identification. The community retrieved from one dung pat in one successional point was considered as one sample in further analyses.

Ecological group delimitation

The dipteran species were classified according to their trophic, primarily larval, specialization. This classification is based upon the same principles as in our previous study of coprophilous beetles (Sladecek *et al.* 2013). The fly species were classified as: (i) coprophages, which are species whose both adult and larvae feed solely, or predominantly, on dung or other decaying matter (most species, e.g. *Musca autumnalis* De Geer) (Skidmore 1985; Haenni 1997; Pape 1998; Papp 1998; Papp & Wheeler 1998; Rognes 1998; Gregor *et al.* 2002); (ii) omnivores, which are predatory in one stage of their development, or can not finish their development without consumption of living food, or are known to regularly prey on other species (e.g. *Myospila meditabunda* (Frabricius)) (Hammer 1941; Laurence 1954; Skidmore 1985; Blackenhorn *et al.* 2010; Dickson *et al.* 2012); and (iii) predators, which are predatory in all stages of development (e.g. Dolichopodidae, Empididae) (Cumming & Cooper 1993; Hulcr *et al.* 2005).

The coprophages were additionally classified as: (i) relocators, whose larvae feed on other decaying matter than dung, and thus relocate larvae from dung pats (but adults visit dung pats regularly for their protein nutrition, e.g. Calliphoridae spp.) (Erzinclioglu 1996); and (ii) dwellers, whose larvae develop directly in the dung pats (most coprophagous species) (Skidmore 1985; Rognes 1998; Gregor *et al.* 2002).

Statistical analyses

Temporal trends at the whole community level

The adult Diptera abundances and species richness trends were analyzed in our data along successional and seasonal gradients by generalized linear models (GLM) computed in Canoco for Windows 5 (Ter Braak & Smilauer 2012). For both abundances and species richness, a GLM with Poisson error distribution was fitted with either succession or seasonality as predictor. The polynomial degree of model fitted (linear or quadratic) was chosen according to the Akaike Information Criterion (AIC).

Since it was not possible to identify all individuals into species, we analyzed the species richness at three levels: (i) species richness as the number of properly identified species (e.g. *Scathophaga stercoraria* (Linnaeus)) per pat; (ii) the number of clearly identified species per pat plus the number of morphospecies (e.g. Chironomidae spp.); and (iii) the number of morphospecies only. Each morphospecies (i.e. morphogenera or morphofamily) was considered as a single species for purposes of analyses.

Temporal trends among individual species of coprophilous Diptera

The successional turnover of coprophilous dipteran adults was analyzed by canonical correspondence analysis (CCA) in Canoco for Windows 5 (Ter Braak & Smilauer 2012). CCA is a multivariate technique suitable for a dataset with species unimodal responses to the environmental gradient (Leps & Smilauer 2003). Multivariate analyses are superior to unimodal methods when testing the response of species-rich communities to the environmental gradients (Leps & Smilauer 2003). Individual species data were $\log(x + 1)$ transformed prior to analysis. Time in hours after placing the dung pat was used as a continuous environmental predictor with sampling season and the affinity to one of the five replications (one of five dung pats in each season), as block covariables. The significance of the successional time on the assemblage of dipteran species on a dung pat was tested by Monte Carlo permutation (999 permutations).

A similar procedure was applied to analyze the seasonal segregation, with season as a factorial environmental predictor (with individual seasons, e.g. early spring, as factor levels) and time, and the affinity to one of the five replications as block covariables.

After these analyses, species were classified into successional and seasonal groups. Species affinity to the seasonal groups was assessed by computing the distance between species symbol and symbols of individual seasons in the ordination diagram of seasonal CCAs, where the species belonged to the season with shortest distance from the species' symbol. Species affinity to the successional groups was assessed by hierarchical clustering in R 2.15.2 (R Core Team 2013) software. Species were clustered by their position on the first canonical axis (representing the succession) in the CCA of successional segregation.

RESULTS

Sampling results

In total, 13579 coprophilous dipteran adults were sampled. We identified 30 species and distinguishable morphospecies (13266 individuals) and three morphogenera where species identification was not possible (*Meoneura*, *Empis*, *Sarcophaga*; 40 individuals); the rest of material was merged as representatives of their families (Anthomyiidae, Syrphidae, Dolichopodidae, Chironomidae, Scatopsidae, Sciaridae, Chloropidae; 273 individuals) (Table 1).

Two blood-sucking species, *Stomoxys calcitrans* (Linnaeus) and *Haematobia irritans* (Linnaeus), which are present infrequently only in the later phase of succession, were excluded from the dataset because their presence was presumably an artifact of an investigator's endothermy.

Temporal trends at the whole community level

Both the dipteran community's per-pat abundances (F = 3081.0, $P < 10^{-6}$) and per-pat species richness, in terms of properly identified species (F = 246.9, $P < 10^{-6}$) and identified species including morphospecies (F = 206.1, $P < 10^{-6}$), decreased over the course of succession with highest values around 1 h of pat age, reaching the maximum of 192 individuals and 12 species (mean per pat abundance: 64.0 ± 9.5 SEM, mean per-pat number of identified species: 8.7 ± 0.5 SEM, mean per-pat identified species plus morphospecies: 9.6 ± 0.5 SEM) (Fig. 1A). Morphospecies alone did not display a significant trend (F = 2.7, P = 0.07). Both the community abundance and species richness then steeply decreased until 72 h of dung pat age (mean per pat; abundance: 14.7 ± 3.5 SEM, identified species: 3.6 ± 0.4 SEM, identified species plus morphospecies: 4.3 ± 0.4 SEM). Afterwards, the abundances and species richness decreased less steeply until 192 h of dung pat age (mean per pat; abundance: 1.2 ± 0.3 SEM, identified species: 0.7 ± 0.20 SEM, identified species plus morphospecies: 1.0 ± 0.2 SEM). The 192 h-old pats were inhabited by several dipteran adults in summer (32 individuals in total) and very sporadically in spring (1 individual) and autumn (3 individuals).

At the seasonal scale, both per-pat abundances (F = 1457.0, $P < 10^{-6}$) and species richness, again as properly identified species (F = 19.9, $P < 10^{-6}$) and identified species including morphospecies (F = 15.6, $P < 10^{-6}$), displayed a numerical affinity to the summer seasons. Both abundances (mean per pat; abundance: 51.9 ± 4.2 SEM) and species richness (the clearly identified species: 6.2 ± 0.3 SEM, identified species including morphospecies: 7.1 ± 0.3) were highest in peak summer (Fig. 1B,C). Morphospecies alone did not display any seasonal trend (F = 0.6, P = 0.57).

Temporal trends among individual species of coprophilous Diptera

The community composition of adult coprophilous Diptera was significantly affected by the age of the dung pat (F = 14.7, P = 0.001; first canonical axis explains 3.5% of variability from 6.62% explainable). All species and morphospecies reached their optima before 96 h (i.e. fifth day) of dung pat age. Along the successional gradient, the whole community was separated by CCA and clustered into two large and four smaller successional groups: (i) early successional group, which generally contained species with optima between the first and second days (1–30 h); and (ii) late successional group with species having their optima between the second and fourth days (30–96 h) (Fig. 2A). Those two large groups can be further classified as four sub-groups: (i) very early successional species presented almost exclusively during the first few hours (1–7 h) of dung existence (e.g. *M. autumnalis*, *Copromyza equina* Fallén, *Hebecnema vespertina* (Fallén)); (ii) early-mid successional species with optima between the first (1–7 h) and second (24–30 h) days (e.g. *My. meditabunda*, *Sepsis* spp., *Empis* spp.); (iii) mid-late successional species with optima between the second (30 h) and third days (48 h) (e.g. *Meoneura* spp. *Coproica* spp.); and (iv) very late

Table 1 List of species sampled, their abundances and guild and temporal group affiliations

	Abbreviation	Guild	Successional group	Season	Sum
Anthomyiidae spp.	A	Dwellers	Late-mid	Late spring	98
Calliphoridae					
Lucilia casear (Linnaeus)	*LC*	Relocaters	Early-mid	Late summer	428
Carnidae					
Meoneura spp.	*Meoneura*	Dwellers	Late-mid	Late spring	29
Dolichopodidae spp.	*D*	Predators	Very late	Peak summer	15
Empididae					
Empis spp.	*E*	Predators	Early-mid	Late spring	2
Chironomidae spp.	*Chir*	Dwellers	Very late	Late spring	72
Chloropidae spp.	*Chloro*	Relocaters	Very late	Peak summer	25
Milichiidae					
Madiza glabra Fallén	*MadGla*	Dwellers	Late-mid	Late spring	381
Muscidae					
Hebecnema nigricolor (Fallén)	*HN*	Relocaters	Very late	Late spring	20
Hebecnema umbratica (Fallén)	*HU*	Omnivores	Early-mid	Early spring	103
Hebecnema vespertina (Fallén)	*HV*	Relocaters	Very early	Early spring	209
Mesembrina meridiana (Linnaeus)	*MeM*	Omnivores	Very early	Late spring	13
Musca autumnalis De Geer	*MA*	Dwellers	Very early	Peak summer	306
Myospila meditabunda (Fabricius)	*MM*	Omnivores	Early-mid	Autumn	23
Sarcophagidae					
Ravinia pernix (Harris)	*R*	Dwellers	Late-mid	Peak summer	12
Sarcophaga spp.	*Sarc*	Relocaters	Late-mid	Peak summer	9
Scatophagidae					
Scatophaga stercoraria (Linnaeus)	*ScatSter*	Omnivores	Very early	Autumn	5
Scatopsidae spp.	*Scatop*	Dwellers	Very late	Autumn	49
Sciaridae spp.	*Sciar*	Dwellers	Very late	Late spring	9
Sepsidae					
Saltella nigripes Robineau-Desvoidy	*SaltNig*	Dwellers	Early-mid	Peak summer	221
Saltella sphondylii (Schrank)	*SaltSpon*	Dwellers	Early-mid	Peak summer	220
Sepsis cynipsea (Linnaeus)	*SC*	Dwellers	Early-mid	Late summer	1174
Sepsis duplicata Haliday	*SepDup*	Dwellers	Late-mid	Late spring	2385
Sepsis punctum (Fabricius)	*SP*	Dwellers	Early-mid	Autumn	238
Sepsis thoracica (Robineau-Desvoidy)	*S*	Dwellers	Early-mid	Peak summer	627
Sphaeroceridae					
Chaetopodella scutellaris (Haliday)	*CS*	Dwellers	Early-mid	Late summer	2826
Coproica acutangula (Zetterstedt)	*CAcu*	Dwellers	Late-mid	Late summer	3575
Coproica ferruginata (Stenhammar)	*CF*	Dwellers	Late-mid	Autumn	49
Copromyza equina Fallén	*CoEqi*	Dwellers	Very early	Early spring	21
Crumomyia nigra (Meigen)	*CN*	Dwellers	Late-mid	Early spring	14
Ischiolepta denticulata (Meigen)	*ID*	Dwellers	Early-mid	Late spring	13
Lotobia pallidiventris (Meigen)	*LP*	Dwellers	Late-mid	Peak summer	40
Lotophila atra (Meigen)	*LA*	Dwellers	Late-mid	Autumn	339
Sphaerocera curvipes Latreille	*SphCur*	Dwellers	Very early	Early spring	8
Stratiomyiidae					
Microchrysa flavicornis (Meigen)	*MicFlav*	Dwellers	Early-mid	Peak summer	8
Sargus flavipes Meigen	*SargFla*	Dwellers	Very early	Autumn	8
Syrphidae spp.	*Syrphid*	Dwellers	Very late	Peak summer	5

Abbreviation, abbreviation used in ordination diagram in Figure 2; Guild, ecological guild where the species/taxon belongs (dwellers: larvae develop in dung pats; relocators: larvae develop out of the dung pat; omnivores: switching between predatory and coprophagy during development; predators: both larva and adult predatory); Successional group, (very early, 1–7 h; early-mid, 1–30 h; late-mid, 30–48 h; very late, 48–96 h); Season, most abundant season (early spring, April–May; late spring, May–June; peak summer, July; late summer, August–September; autumn, September–October); Sum, sum of all individuals from all seasons.

successional species with optima from the third (48 h) to the fourth days of succession (96 h) (e.g. *Hebecnema nigricolor* (Fallén), Chironomidae spp. Scatopsidae spp.) (Table 1). Among ecological groups, the omnivores were all early successional (belonging to the very early and early-mid successional groups). The exclusively predatory

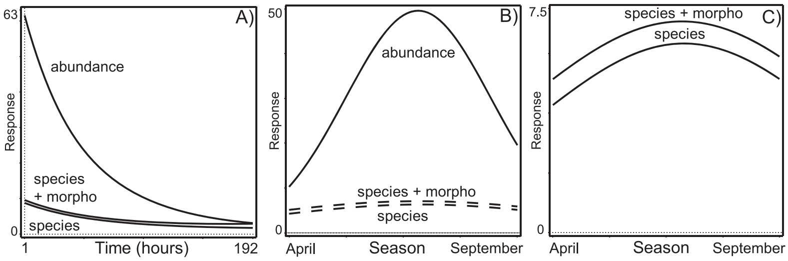

Figure 1 Temporal trends in dipteran adult abundances and species richness. All displayed curves represent the polynomial generalized linear models. Those curves are fitted for the whole community per pat abundance and species richness during succession (A) and season (B,C). Broken curves in (B), representing the species richness as the clearly identified species and the clearly identified species including morphospecies, are plotted for better visualization in (C). Abundance, overall abundance per dung pat; species, number of clearly identified species per dung pat; species + morpho, sum of clearly identified species and morphospecies per dung pat.

species occurred both in early-mid and very late phases of the succession (*Empis* spp. in early-mid and Dolichopodidae spp. in the very late successional groups). The coprophagous relocators were evenly distributed along the successional gradient with the most numerous species, *Hebecnema vespertina*, present in the very early successional group. Most dweller species occurred in the early- and late-mid successional group between 1 and 48 h of dung pat age. From the taxonomical perspective, the succession can be characterized as predominantly Calyptratae: Diptera – Acalyptratae: Diptera – Nematocera. Such classification reflects the individual successional groups, since the very early successional group was mostly formed of Muscidae: Muscinae (*Mesembrina meridiana* (Linnaeus)) and Muscidae: Mydaeiinae (*Hebecnema umbratica* (Fallén), *My. meditabunda*, *He. vespertina*), the early- and late-mid successional groups were mostly formed of extremely abundant representatives of Sepsidae, Carnidae, Milichiidae and Sphaeroceridae (mostly representatives of Limosininae subfamily), and the late successional group was dominated by Chironomidae, Sciaridae and Scatopsidae.

The community composition was also significantly affected by the season ($F = 11.1$, $P = 0.001$; all canonical axes explain 10.1% of variability from 23.94% explainable). Grouping based on species optima in the ordination diagram separated the community into five seasonal groups, each representing one season: (i) early spring group (e.g. *He. vespertina*, *He. umbratica*); (ii) late spring group (e.g. Chironomidae spp., *Sepsis duplicata* Haliday); (iii) peak summer group (e.g. *Saltella* spp., *M. autumnalis*, Dolichopodidae spp.); (iv) late summer group (e.g. *S. cynipsea* (Linnaeus), *Lucilia caesar* (Linnaeus)); and (v) autumn group (e.g. *My. meditabunda*, *S. punctum* (Fabricius)) (Table 1). Those five groups could be simplified as the spring (early spring species), autumn (autumn species) and summer (both peak and late summer species) groups (Fig. 2B). Late spring was a more transitional season, with some species rather inclining to the spring season (Chironomidae spp., *Mesembrina meridiana*, Anthomyiidae spp. and Sciaridae spp.), while other (e.g. *Empis* spp. *Meoneura* spp.) were rather inclined towards the summer season. Regarding ecological groups, the omnivores displayed a strong affiliation to the spring–autumn seasons, while most coprophages (both relocators and dwellers) displayed a preference for the summer seasons. The dwellers present in spring–autumn were generally either late successional (Chironomidae, Scatopsidae, Sciaridae), or very early successional (*Copromyza equina*, *Lotophila atra* (Meigen), *Sphaerocera curvipes* Latreille). The only relocator species to display a spring–autumn seasonality was again a member of the early successional group (*He. vespertina*). The predators were present predominantly in summer, with *Empis* spp. having an optimum in late spring but rather inclining towards the summer. From the taxonomic perspective, all Nematocera groups, and Muscidae: Mydaeinae (except for *He. nigricolor*), displayed a numerical preference for the spring (or late spring in Chironomidae and Sciaridae) and autumn. Most spring and autumn species belonged to the family Sphaeroceridae subfamilies Copromyzinae and Sphaerocerinae.

DISCUSSION

Temporal trends at the whole community level

The successional process in the cow dung surface community can be characterized as a rapid decline of both community abundance and species richness. This decline is however not as steep as previous studies predicted.

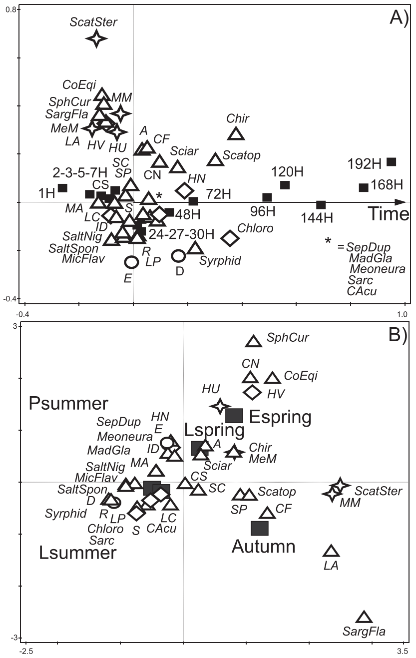

Figure 2 Canonical correspondence analysis ordination diagrams of temporal trends in coprophilous dipteran adult communities. (A) Succession of dipteran adults (F = 14.7, P = 0.001; first canonical axis explains 3.5% of variability from 6.62% explainable). (B) Seasonality of dipteran adults (F = 11.1, P = 0.001; all canonical axes explain 10.1% of variability from 23.94% explainable). Filled squares represent individual successional points, plotted as supplementary variables, in (A) or individual sampling seasons in (B). Empty symbols represent individual species. Shapes of symbols reflect the particular species trophic/larval ecology: triangle, dwellers; diamond, relocators; star, omnivorous species; circle, predatory species. Espring, early spring; Lspring, late spring; Psummer, peak summer; Lsummer, late summer; Autumn, autumn. A, Anthomyiidae spp.; CAcu, *Coproica acutangula*; CF, *Coproica ferruginata*; Chir, Chironomidae spp.; Chloro, Chloropidae; CN, *Crumomyia nigra*; CoEqi, *Copromyza equina*; CS, *Chaetopodella scutellaris*; D, Dolichopididae spp.; E, *Empis* spp.; HN, *Hebecnema nigricolor*; HU, *Hebecnema umbratica*; HV, *Hebecnema vespertina*; ID, *Ischiolepta denticulata*; LA, *Lotophila atra*; LC, *Lucilia caesar*; LP, *Lotobia pallidiventris*; MA, *Musca autumnalis*; MadGla, *Madiza glabra*; Meoneura, *Meoneura* spp.; MeM, *Mesembrina meridiana*; MicFlav, *Microchrysa flavicornis*; MM, *Myospila meditabunda*; R, *Ravinia pernix*; S, *Sepsis thoracica*; Sarc, *Sarcophaga* spp.; SargFla, *Sargus flavipes*; SaltNig, *Saltella nigriceps*; SaltSpon, *Saltella spondylii*; SC, *Sepsis cynipsea*; ScatSter, *Scathophaga stercoraria*; Scatop, Scatopsidae spp.; Sciar, Sciaridae spp.; SepDup, *Sepsis duplicata*; SP, *Sepsis punctum*; SphCur, *Sphaerocera curvipes*; Syrphid, Syrphidae spp.

The most detailed studies on succession of a cattle dung surface community so far, Hammer (1941) and Mohr (1943), observed dipteran adults colonizing dung pats, culminating in a matter of minutes or hours. After this culmination, most of the species reportedly disappeared before the dung pat reached 24 h in age. The only exceptions were Sepsidae and Sphaeroceridae, whose representatives stayed on the dung pats up to several more days. Therefore, our results differ from the previous findings: although both the community abundances and species richness peaked during the first few hours, the community still contained more than one-third of the maximum abundance and species richness 72 h after the dung pat was created. The surface community existed generally for eight days or, in a much-reduced form, even more days. This temporal scope is partly in agreement with Mohr's (1943) observation that the surface community lasted 5–8 days depending on dung pat moisture. It is important to note that our study and both the former studies targeted the dipteran community visiting cow dung. Successional patterns on the dipteran community visiting horse dung also display a steep decline in abundance during the succession, but the surface community does not last for more than 24 h after the dung placement (Psarev 2002), likely because horse dung is generally much drier than cow dung (Mroczunski & Komosinski 2014).

The dynamics of the dipteran community on carrion are also characterized by a decline throughout the succession (e.g. Tabor *et al.* 2005; Matuszewski *et al.* 2011). Although only semi-quantitative data exist, this decline is probably slower, especially on large carrion.

Both the species richness and abundance were highest between early and late summer. This agrees with Hammer's (1941) and Mohr's (1943) observations that most of the dipteran adults visiting cow dung were active during warm summer months. Similar patterns were also reported from carrion communities. Here, the most abundant Calliphoridae are most active during warm weather (George *et al.* 2013), i.e. during summer.

Temporal trends among individual species of coprophilous Diptera

Although both canonical correspondence analyses, for succession and seasonality, were significant, one could argue that neither of them explained a convincing amount of variability (3.5% for succession, 10.1% for season). However, the maximum amount of explainable variability in multivariate analyses is usually not 100% (6.62% for succession and 23.94% for season in our study) (Leps & Smilauer 2003). So, our analyses explained almost 53%

for succession and 42% of potential variability in the community of adult coprophilous Diptera.

The succession of adult dipterans on dung pats has always been documented as patterns of colonization, emphasizing early arrival and quick departure for most species, except for Sepsidae and Sphaeroceridae families (Hammer 1941; Mohr 1943). However, we found that a vast majority of species had their optima between the first and second days of dung pat existence, rejecting earlier claims that most of the species stay at the dung pat for just several minutes. Instead, no species had a distinct optimum on 1 hour-old pats.

The difference between our results and Hammer (1941) or Mohr (1943) might have been caused by different methodologies. Those authors observed the activity of dipteran individuals, who are indisputably able to spend only several minutes on dung pats. On the contrary, we based our study on capturing the whole communities in specific time intervals, which enabled us to more precisely assess the optima of individual species.

Despite the different approaches, the individual species' successional sequence that we observed much resembled sequences described by our predecessors (Hammer 1941; Mohr 1943). In the former studies, the succession generally followed the pattern of change from Calyptratae to Acalyptratae: Diptera, mostly due to the very early arrival of Calyptratae: Diptera species on dung pats. This pattern also applied in our study, in which the Calyptratae: Diptera were present during the earliest phases of succession, whereas the Acalyptratae: Diptera were present throughout the succession up to a point when they were replaced by Nematocera, namely the representatives of Chironomidae, Scatopsidae and Sciaridae. This result is in agreement with Mohr's (1943) prediction that the successional turnover proceeds from specialists to generalists.

Similar patterns of species specialization and taxa replacement during succession exist also in the carrion community. On carrion, the earliest successional colonizers are Calliphoridae (Calyptratae), using carrion almost exclusively as breeding habitat (Rognes 1998). They are followed by Sepsidae and Piophilidae, both Acalyptratae and both habitat generalists (Grassberger & Frank 2004; Tabor *et al.* 2004; Matuszewski *et al.* 2011).

Regarding ecological guilds, only one ecological guild displayed compact successional optima. The omnivores were all early successional, attributably due to their larval habit of preying on larvae of other dipteran groups, predominantly the Calyptratae fly larvae (which are also early colonizers) (Skidmore 1985; Dickson *et al.* 2012). The exception is *Scatophaga stercoraria*, whose adults are predatory and larvae coprophagous (Blanckenhorn *et al.* 2010). Nevertheless, the very early successional appearance of their adults matches the highest abundance of other dipteran species, their prey. The predators were mostly abundant in the very late succession (Dolichopodidae spp.) and had some presence in the early-mid succession (*Empis* spp.). In both cases, they matched the high abundance of smaller Acalyptratae and Nematocera species, their prey (Cumming & Cooper 1993). The other two guilds, relocators and dwellers, occurred throughout the successional gradient, with the most abundant relocators occurring early in succession. It is not possible to compare communities visiting dung and carrion, since all dipteran species in carrion behave as dwellers.

Contrary to the former studies, we did not observe a significant colonization by blood-sucking *H. irritans*, an early dung visitor (Hammer 1941; Mohr 1943). This species colonizes fresh dung immediately after defecation by cattle, on which it perches at that moment (Kuramochi 2000). Our dung pats did not attract this species, although *H. irritans* is unlikely to influence the further fate of the surface coprophilous community.

The coprophilous Diptera had two seasonal components: the spring–autumn group and summer group, as again observed by both Hammer (1941) and Mohr (1943). Most of the coprophilous dipteran species had their optima between late spring (May–June) and summer. Also, in the carrion dipteran community, most species occur in summer (Matuszewski *et al.* 2010; Benbow *et al.* 2013). The spring–autumn community is composed of species that are either early successional (e.g. *He. umbratica*) with optima during the first hours of dung pat existence, or late successional (e.g. Chironomidae spp.), with optima after 48 h of dung pat existence.

Among coprophagous species, this pattern can be attributed to larval dynamics, since larvae of species with optima in spring–autumn should occur mainly throughout autumn, winter and spring, thus avoiding the highly abundant larval aggregation in summer (Laurence 1954). Although no competition between larvae of, at least, Calyptratae: Diptera was predicted by Valiela (1969), the large abundance of summer species' larvae, especially those of early successional Calyptrate: Diptera, can destroy the dung pat by shredding it, thus depriving later occurring Acalyptratae and Nematocera species of their habitat (Wu & Sun 2010). The other possible explanation is based on environmental conditions. Since the temperatures are lower during spring and autumn, the dung pat dries slowly, allowing for the existence of the late-successional species. The same explanation could be drawn for omnivores, since the Muscidae: Mydaeinae (*He. umbratica* and

My. meditabunda) and *Sc. stercoraria* are negatively affected by higher temperatures and therefore occur primarily during cooler spring and autumn (Hammer 1941; Blanckenhorn *et al.* 2010; Kruger *et al.* 2010).

Even though most coprophagous species are most abundant in summer, and therefore potentially subjected to larval competition, the individual species display a certain pattern of separation. The most obvious is seasonal microseparation among species-rich Sepsidae in which such segregation was predicted (Rohner *et al.* 2015). The individual species of Sepsidae occur almost together in the succession and their larvae should appear at similar successional times (Laurence 1954). However, the seasonal optima of individual species are separated, forming a continual line of species displacements (i.e. *S. duplicata* in late spring, *Saltella* spp. and *S. thoracica* (Robineau-Desvoidy) in peak summer, *S. cynipsea* in late summer and *S. punctum* in autumn). The predatory species were most abundant during summer, together with highest abundance of their potential prey, the Acalyptratae: Diptera.

CONCLUSIONS

To the best of our knowledge, our study represents the first attempt to quantitatively analyze the occurrence of dipteran adults along successional and seasonal gradients on cattle dung. The species' temporal sequences retrieved in our study, both during succession and season, resemble the sequences retrieved in earlier studies. Both the abundance and species richness decreased with increasing dung pat age, but the decrease was less steep than claimed by other authors. The community was separated into two major groups (early and late) and four subgroups along the successional gradient: (i) species occurring during the first few hours; (ii) species occurring between 1 h and 30 h of dung pat existence; (iii) species with optima between 30 h and 48 h of dung pat existence; and (iv) species with optima between 48 h and 96 h of dung pat existence. The species with early and late successional optima occurred during spring–autumn and therefore were seasonally separated from species from the mid-successional group, which prevailed during summer. This separation was either due to niche separation between larvae or to tolerance to lower temperatures in spring and autumn.

We are aware of potential problems of this study with species identification in several taxonomically difficult families (e.g. Chironomidae). Because of this, we used several alternative versions for the species richness calculation, which nevertheless performed almost identically.

We focused exclusively on the temporal patterns displayed by coprophilous dipteran adults. To obtain a full picture and explanation of the patterns observed, the results should be combined with studies of dipteran larvae and coprophilous beetles. Only such multitaxa analysis will fully interpret the temporal patterns in coprophilous insects.

ACKNOWLEDGMENTS

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 are also very grateful to Jindrich Rohacek for advice during species identification. We thank Tomas Zitek, Petr Sipek and Simon T. Segar for comments on the early version of the manuscript and to Matthew Sweney for language correction. 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 the University of South Bohemia (152/2016/P).

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CHAPTER II

Frantisek Xaver Jiri Sladecek, Simon Tristram Segar, Colin Lee, Richard Wall, Martin Konvicka, 2017. *Temporal segregation between dung-inhabiting beetle and fly species.* PLoS ONE 12(1): e0170426.

RESEARCH ARTICLE

Temporal Segregation between Dung-Inhabiting Beetle and Fly Species

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Abstract

The coexistence of ecologically similar species (i.e. species utilizing the same resource) is a major topic in ecology. Communities are assembled either through the biotic interactions of ecologically similar species, e.g. competition, or by the abiotic separation of species along gradients of environmental conditions. Here, we investigated the temporal segregation, succession and seasonality of dung-inhabiting Coleoptera and Diptera that utilize an identical resource in exactly the same way. The data were collected from two temperate pastures, one in the United Kingdom and the second in the Czech Republic. There was no evident temporal separation between ecologically similar coleopterous or dipterous taxa during succession. In contrast, these two orders were almost perfectly separated seasonally in both combined and site-specific datasets. Flies were most abundant in the summer, and beetles were more abundant in the spring and autumn. Ecologically similar beetles and flies also displayed seasonal separation in both combined and site-specific data. Analyses within site-specific data sets revealed such a separation at both the order and species level. Season is therefore the main temporal axis separating ecologically similar species of dung-inhabiting insects in temperate habitats, while succession aggregates species that may have similar environmental tolerances (to e.g. dung moisture). This separation between ecologically similar taxa of beetles and flies may be attributable to either competition-based niche separation or to temperature tolerance-based habitat filtering, since flies have peak activity in warmer months while beetles have peak activity in cooler months.

Citation: Sladecek FXJ, Segar ST, Lee C, Wall R, Konvicka M (2017) Temporal Segregation between Dung-Inhabiting Beetle and Fly Species. PLoS ONE 12(1): e0170426. doi:10.1371/journal.pone.0170426

Editor: Heike Lutermann, University of Pretoria, SOUTH AFRICA

Received: September 16, 2016

Accepted: January 4, 2017

Published: January 20, 2017

Data Availability Statement: All data files are available from the Figshare repository (doi: dx.doi.org/10.6084/m9.figshare.3823959.v1).

Funding: This work was supported by the Institute of Entomology, Biology Centre of the Czech Academy of Science (RVO//:60077344); Grant Agency of University of South Bohemia (152/2016/P); Studentship funding from the University of Bristol, UK; Simon T Segar acknowledges funding from and a Univ. of South Bohemia Postdoc project (reg.no. CZ.1.07/2.3.00/30.0006) (funded by the EU Education for Competitiveness

Introduction

One of the most important questions in ecology is why there are communities and not single species assemblages [1]; the coexistence of species rich communities has been a topic of major research interest in ecology.

Natural communities are assembled, and species coexistence is facilitated, by two contrasting processes: niche differentiation and habitat filtering [2–4]. Niche differentiation separates species with similar traits and promotes the coexistence of ecologically similar species via their

Operational Programme, the European Social Fund and the Czech State Budget). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

segregation [3, 5, 6]. Such segregation usually takes place along resource, habitat and temporal axes [7]. Habitat filtering aggregates species with similar traits, which in turn must have some tolerance to the environmental factors, such as temperature and humidity, etc. [2, 4, 6]. In addition to such ecological patterns, community assembly can also be detected in the phylogenetic structure of a community: if related species with similar traits are clustered it suggests habitat filtering; and if related species with similar traits are dispersed, it suggests niche differentiation [8, 9]. Although both habitat filtering and niche differentiation are quite extensively studied, there is still no consensus on the relationship between the mechanisms, nor which one is generally the most important for the coexistence of species rich communities [10–12]. However, environmental filtering is usually considered to be the main mechanism on large spatial scales, i.e. the assembly of large species rich communities [13–17], while both environmental filtering and niche differentiation are considered to play a role at the smaller spatial scale, e.g. relations between several species within the community [18, 19]. Niche differentiation and habitat filtering have usually been tested in communities present on multiple sites [e.g. 20] as well as in communities along altitudinal [e.g. 15, 21] or temporal gradients [e.g. 12].

Temporal gradients have generally been considered to play a key role in species coexistence (by separating similar competitors), although habitat filtering is probably also involved [5, 7, 12]. Temporal segregations usually involve the daily activity of ecologically similar species in actively foraging animals [18, 19]. Plants and "plant-like" sessile animals are separated along long-lasting successional gradients with competitively inferior species in early succession, and competitively dominant species in late succession, or vice versa [12, 22]. Finally, predominantly insect or invertebrate species coexistence is greatly maintained via their seasonal displacements [23, 24]. A good model system with species temporal patterns along all three gradients (daily activity, succession and seasonality), and in which both habitat filtering and niche separation could apply, are the communities inhabiting ephemeral habitats.

Ephemeral habitats, such as dung, carrion, fruiting bodies of mushrooms or rotten fruits, are temporally unstable, spatially and temporally random, yet usually provide a very high energy content for their inhabitants [25]. These inhabitants include fungi, bacteria and several animal groups, predominantly arthropods, nematodes and annelids [26, 27]. Temporal patterns of colonization of such habitats have been studied almost exclusively in insect representatives of Arthropods (all occupancy patterns). The temporal segregation among insects, at all three levels, is traditionally considered to be associated with niche differentiation due to instability and the limited mass of their primary resource [28, 29]. However, there is evidence for potential habitat filtering: for succession, for example mediated through dung moisture tolerance [30], and on the seasonal scale, for example mediated through temperature [31, 32] or drought tolerance [33]. The majority of such evidence comes from the insect community inhabiting the dung, which is the focus of the current study.

Competition and coexistence, based on niche differentiation in time, is very often cited for maintaining communities of dung-inhabiting (coprophilous) insects along temporal gradients [34]. However, the vast majority of studies that predict niche differentiation, especially in temperate communities, have been carried out solely using coprophilous beetles as the model communities, disregarding coprophilous flies [e.g. 24, 35]. Without flies–the second most prominent dung-inhabiting insect group–it is not possible to correctly assess whether the beetle temporal patterns are really due to their suggested niche differentiation with flies [36], or due to habitat filtering based upon dung pat properties for succession [e.g. 37].

The temporal patterns of beetles have been studied extensively and quantitatively both in temperate [e.g. 24, 37] and sub-tropical and tropical regions [e.g. 28, 38]. However, in contrast to beetles, fly temporal patterns have been studied quantitatively only infrequently [39, 40] and in a rather qualitative manner only [41, 42].

Here we therefore present a study focused on both dung-inhabiting beetles and flies, including both adults and larvae. We studied the two most prominent temporal segregations in temperate communities, succession and seasonality, using data from two sites, Central Europe [24, 40] and the United Kingdom [39]. We investigated: A) temporal patterns of the coprophilous beetles and flies as whole taxonomic groups, and B) temporal patterns of similar functional groups of coprophilous beetles and flies. Based on these patterns we further assessed whether the taxonomic or functional groups display niche differentiation (avoiding each other along the temporal gradient, with their model-fitted curves non-overlapping) or habitat filtering (co-occurrence along the temporal gradient, with their model-fitted curves overlapping).

Material and Methods

Study sites

The study was carried out on two pastures; one situated 10 km west of Ceske Budejovice, Czech Republic (CZ); and one situated 20 km south-west of Bristol, United Kingdom (UK).

Both pastures hosted a permanent herd of adult cows and had been continuously grazed in previous years. The CZ site 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. The UK site is situated 100 m.a.s.l., in a region with a mean annual temperature of 11˚C, mean annual precipitation of 850 mm. The UK site represents an oceanic climate with cooler summers and cool, yet not cold winters; the CZ site represents a continental climate with warm summers and colder winters. At both sites, the highest temperatures occur during the summer months (June–August) and the vegetation season spans from early spring (March–April) to autumn (October).

No official permit was required to carry the study on neither of study sites, as both are personal property of their owners. No special permit was needed to work with studied animals, as we worked with insects. One CZ beetle species, *Emus hirtus* (Linnaeus, 1758), is considered endangered in Czech Republic, however, we specifically did not killed individuals of this species (and immediately released them), as this species is easy to identify even in field.

Insect sampling

At both sites, insect sampling was conducted using artificially created dung pats. Pats of 1.5 litres in volume were used in CZ, and pats of 1.5 kg of wet weight in the UK. The fresh, just defecated, dung was gathered from permanently stalled cows in CZ, and from pasture grazed cows after milking in the UK. Dung was thoroughly mixed and homogenized before exposition. The dung pats used for sampling were then created at the study sites. Following [43], we presume that there might be some minor differences between communities in dung from pasturing cows and communities in dung from cows fed on hay or silage, but no insect species was found to be exclusive to one of those types and super-abundant species were super-abundant in both such dung types [43]. We also presume that the artificially created dung pats should not substantially differ in their insect communities from pats naturally dropped by cows [43].

The CZ insect data were collected between 2009 and 2012 as part of three separate projects. The Coleoptera were sampled five times per vegetation season (11–29 April, 17 May– 4 June, 4–22 July, 15 August– 2 September and 23 September– 11 October) in 2009. During those seasons, the beetles were sampled from dung pats aged 1, 2, 3, 4, 5, 7, 9, 11 or 14 days. Each successional time was represented by one unique dung pat in each season.

Each successional time was replicated five times per season. So one replication consisted of a nine dung pats placed as a line in the field (nine successional times) with pats 5 m apart.

The position of individual successional pats was randomized within this line. On the next day, another line (the second replication) was laid 5m apart from the first line (the first replication). On the third day three more replications were laid, forming a total of five replications per sampling season [24].

Adult Diptera were sampled in six sampling seasons: three in 2011 (23 April– 1 May, 16–24 July, 26 August– 3 September) and three in 2012 (9–17 May, 27 July– 4 August 2012, 14–22 September). The adult dipteran community was sampled at 1, 2, 3, 5, 7, 24, 27, 30, 48, 72, 96, 120, 144, 168 and 192 hours of dung pat age (first day, 1, 2, 3, 4, 5, 7 and 8 days of dung pat age). This sampling was conducted from five dung pats (each representing one replication per sampling season) [40].

Larval Diptera and Coleoptera were sampled three times in 2011 (18 April– 3 May, 12–27 July and 22 August– 6 September), from the pats aged 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 days, each replicated four times per sampling season with identical pattern of replicate lines being laid on successive days as for CZ adult beetles. Data on fly larvae come from an unpublished study currently under review.

The CZ Coleoptera and larvae were sampled by floating the dung pat and the portion of underlying soil in a bucket of water. The CZ adult flies were sampled by rapidly covering the dung pat by a sweeping net, catching the disturbed fly individuals perching on the dung pat's surface.

The UK data were collected in 2001 over a period of 24 weeks (the first week starting on 21 May, the last one on 29 October). At the start of each week, 10 dung pats were created and exposed in a pasture for 7 days, after which the pats were taken to the laboratory for insect extraction. Each dung pat was put in a fine-meshed bag and left 10 weeks to allow for the emergence of insects. As they emerged, live insects were funnelled into a collecting pot, to prevent the re-colonization of pats [39].

More details on the data sampling are provided in the respective publications [24, 39, 40].

Functional groups and guilds

In addition to analyzing the raw numbers of beetles and flies, both beetle and fly species were classified into functional groups. For this, insect individuals were identified as further into species level as it was possible. Whenever species identification was not possible, we tried to establish the morpho species at lowest taxonomic level possible, which allowed for proper ecological classification.

The beetle and fly species were sorted primarily into three major functional groups: 1) the strict saprophages (i.e. coprophages), whose adults and larvae both utilise decaying matter or do not require living food to finish their development [44, 45]; 2) the omnivores, species which shift from predation to coprophagy (or vice versa) during their development [44, 46] or species that are not able to complete their development without living food even though their other development stages are coprophagous [45]; and 3) the predators, who utilise solely living prey when both larvae and adults [44, 47].

The saprophages could be further separated into two guilds that differ in their resource utilisation. Those are: i) the relocators, whose larvae do not live in the dung pat [24] and either live in underground nests prepared by their parents [48], or whose larvae utilise other kinds of decaying matter whilst their parents visit the dung pats solely for their own nutrition [49]. The second guild are: ii) the dwellers, whose larvae develop in the dung pat itself in at least one stage of their development. This guild comprises the majority of coprophilous saprophages [45, 49]. A detailed ecological classification of the beetle and fly taxa sampled is provided in the Supporting Information (S1 Table).

Data preparation and taxa selection

The datasets (CZ and UK) were adjusted for two types of analyses; the site-specific analyses to compare the patterns between the two independent European temperate sites (CZ and UK), and one with the combined datasets to present the patterns at a broader temperate scale.

To make datasets from both sites comparable, the UK data were adjusted to the CZ data. The seasonal gradient in the CZ data is based primarily upon "seasons", i.e. periods of year which are set up loosely at the month level, as starting and end dates are affected by weather conditions (early spring = April–first half of May; late spring = second half of May–June; early summer = July; late summer-early autumn = August–early days of September; and late autumn = second half of September–October). These seasons host a specific, yet predictable community composition (i.e. the dung insect community in early spring 2009 was almost identical to early spring 2011) as the seasonal patterns seem to be stable in both dung-inhabiting beetles [24, 39, 50, e.g. 51] and flies [40–42]. To match this seasonal pattern in the UK data, the weeks that overlapped with sampling seasons in CZ were chosen for future analyses (there were three UK weeks per one CZ sampling season). Since it was not possible to sample the early spring season in the UK, the early spring was also omitted from CZ data for the combined UK and CZ dataset, creating a dataset with 4 seasons. This CZ early spring was however used for analyses of individual sites, since otherwise the CZ site would have only two seasons for larval data. The arrangement of the UK dataset for single-site analyses was identical to that used for the combined data analysis.

The data from both sites, CZ and UK, were used to analyze the seasonal segregations between beetles and flies. However, only the data from the CZ site were used to investigate the successional separations, since the UK data were not sampled as successional lines.

Finally, the flies were represented by their larvae only in analyses along the successional gradient, whereas both fly adults and larvae were used in analyses along the seasonal gradient. This change was necessary because the beetle community, along the gradient of dung pat ageing, is much more likely to interact with fly larvae who inhabit the same interior of the dung pat, rather than with fly adults who perch on the dung pat surface and are most abundant only during the very first hours of dung pat existence [41, 42]. In the same fashion, only larvae of omnivorous beetles were used for analyses of successional segregation, since the vast majority of adult omnivorous beetles are very early successional and therefore do not interact with their larval fly omnivorous counterparts [24].

Statistical analyses

The temporal trends of raw counts of beetles and flies, functional groups and ecological guilds of beetles and flies were analyzed using generalized linear models (GLM) in CANOCO 5 [52]. The response of each investigated group, e.g. beetle saprophages, was chosen as either linear or quadratic by AIC. To avoid the impact of over-dispersion, which is frequently present in GLMs with Poisson distribution of errors, all models were fitted with quasi-Poisson distribution of errors. The fitted GLM curves show us whether the beetle and fly functional groups 1) co-occur along temporal gradients (i.e. habitat filtering) [2], or 2) are separated (i.e. *a priori* niche differentiation) [3].

To further support the temporal trends of beetle and fly functional and ecological groups, we also analyzed the seasonal segregations among individual species of beetles and flies. We have chosen season exclusively, as we lack data on succession for the UK. Seasonal patterns of individual species were analyzed separately for the CZ and UK data with Detrended Canonical Correspondence Analysis (DCCA) in CANOCO 5 [52]. DCCA is a multivariate method suitable for data with unimodal species' responses along the gradient [53]. DCCA also prevents the occurrence of a prominent artifact, the arch effect (which occurred in our data when using non-detrending analysis like Canonical Correspondence Analysis). We used detrending by second order polynomial. Species data were log (x+1) transformed prior to the analyses. The significance of seasons, in the form of factorial variables, was tested by Monte Carlo permutation test (999 permutations).

Results

The entire CZ dataset comprised 58,774 individuals in 107 species and morpho-species, the entire original UK dataset comprised 145,454 individuals in 47 species and morpho-species. Such a huge abundance of insects in the UK data was largely due to one species, *Sylvicola punctata* (Diptera: Anisopodidae), which was *a priori* omitted as an outlier, contributing 92,485 individuals in the UK dataset (64%). The combined dataset from both sites, after adjustments and omitting *S. punctata*, contains 78,036 individuals (28,645 UK; 49,391 CZ–excluding the early spring season) (Table 1). The datasets for individual sites consist of 28,645 individuals for the UK and 58,774 for CZ (including the early spring season).

Since almost all GLMs were significant, we provide the significances and test-values of GLMs testing the dung-inhabiting insects trends in succession and seasonality in Table 2.

Temporal patterns of coprophilous beetles and flies as general taxonomic groups

Beetles and flies displayed significant trends along both the successional and seasonal gradients (Fig 1A and 1B). Both of these temporal gradients, however, strongly differed. Along the successional gradient, the occurrence of beetles and fly larvae almost overlapped, with both groups peaking at practically the same time; while with respect to season beetles and flies displayed a separation in the combined data. Flies reached their maximum abundance during summer, especially late summer, while beetles were most abundant during the spring and autumn seasons.

Table 1. Summary of individual functional group abundances in the combined seasonal data and in the successional data (from Czech Republic).

Beetles	Late spring	early summer	late summer	autumn	sum of seasons	succession (CZ only)
all	12163	6341	10240	9263	38007	39810
saprophages (all)	3117	1140	935	2983	8175	11653
saprophages (dwellers)	1811	1107	830	2846	6594	7537
saprophages (relocators)	1306	33	105	137	1581	4116
omnivores/larvae	6404	4226	7554	4263	22447	218
predators	2642	975	1751	2017	7385	-
Flies	Late spring	early summer	late summer	autumn	sum of seasons	succession (CZ only)
all/larvae	3175	14657	13599	8598	40029	5385
saprophages (all)/larvae	2928	14175	13053	8039	38195	5263
saprophages (dwellers)/larvae	2836	13847	12941	7985	37609	5263
saprophages (relocators)	92	328	112	54	586	-
omnivores/larvae	245	379	544	559	1727	122
predators	2	103	2	0	107	-

Seasons: late spring = second half of May—June; early summer = July; late summer—early autumn = August—early days of September; and late autumn = second half of September—October. Functional groups: all = all beetles or flies, saprophages = both adults and larvae coprophagous (dwellers = larvae develop in the dung pats, relocators = larvae develop outside of the dung pat), omnivores = trophic switch between adults and larvae (usually adult saprophage, larva predator), predator = both adult and larvae predatory).

doi:10.1371/journal.pone.0170426.t001

Table 2. Results of GLMs testing the seasonal and successional trends of coprophilous insects.

data	temporal gradient	order	functional group (guild)	df	*F*-value	*P*-value
combined	season	beetles	all	3 / 760	12.0	$<10^{-5}$
combined	season	beetles	saprophages (all)	3 / 760	42.9	$<10^{-5}$
combined	season	beetles	saprophages (dwellers)	3 / 760	42.5	$<10^{-5}$
combined	season	beetles	saprophages (relocators)	3 / 760	40.6	$<10^{-5}$
combined	season	beetles	omnivores	3 / 760	2.1	0.13
combined	season	beetles	predators	3 / 760	27.4	$<10^{-5}$
combined	season	flies	all	3 / 760	17.1	$<10^{-5}$
combined	season	flies	saprophages (all)	3 / 760	17.8	$<10^{-5}$
combined	season	flies	saprophages (dwellers)	3 / 760	17.9	$<10^{-5}$
combined	season	flies	saprophages (relocators)	3 / 760	7.2	$<10^{-3}$
combined	season	flies	omnivores	2 / 761	16.6	$<10^{-4}$
combined	season	flies	predators	3 / 760	29.4	$<10^{-5}$
CZ	season	beetles	all	3 / 744	4.3	0.01
CZ	season	beetles	saprophages (all)	3 / 744	24.6	$<10^{-5}$
CZ	season	beetles	saprophages (dwellers)	3 / 744	24.4	$<10^{-5}$
CZ	season	beetles	saprophages (relocators)	2 / 745	79.7	$<10^{-5}$
CZ	season	beetles	omnivores	2 / 745	4.5	0.04
CZ	season	beetles	predators	3 / 744	10.3	$<10^{-4}$
CZ	season	flies	all	3 / 744	25.3	$<10^{-5}$
CZ	season	flies	saprophages (all)	3 / 744	25.9	$<10^{-5}$
CZ	season	flies	saprophages (dwellers)	3 / 744	25.3	$<10^{-5}$
CZ	season	flies	saprophages (relocators)	3 / 744	6.5	$<10^{-2}$
CZ	season	flies	omnivores	2 / 745	6.2	0.01
CZ	season	flies	predators	3 / 744	16.0	$<10^{-5}$
UK	season	beetles	all	3 / 117	23.0	$<10^{-5}$
UK	season	beetles	saprophages (all)	2 /118	4.0	0.05
UK	season	beetles	saprophages (dwellers)	2 /118	3.9	0.05
UK	season	beetles	saprophages (relocators)	3 / 117	15.2	$<10^{-5}$
UK	season	beetles	omnivores	3 / 117	28.5	$<10^{-5}$
UK	season	beetles	predators	3 / 117	13.0	$<10^{-5}$
UK	season	flies	all	3 / 117	80.4	$<10^{-5}$
UK	season	flies	saprophages (all)	3 / 117	73.6	$<10^{-5}$
UK	season	flies	saprophages (dwellers)	3 / 117	73.6	$<10^{-5}$
UK	season	flies	omnivores	3 / 117	23.4	$<10^{-5}$
UK	season	flies	predators	3 / 117	48.3	$<10^{-5}$
CZ	succession	beetles	all	3 / 744	5.0	$<10^{-2}$
CZ	succession	beetles	saprophages (all)	3 / 744	5.7	$<10^{-2}$
CZ	succession	beetles	saprophages (dwellers)	3 / 744	10.7	$<10^{-4}$
CZ	succession	beetles	saprophages (relocators)	2 / 745	10.4	$<10^{-2}$
CZ	succession	beetles larvae)	omnivores	3 / 744	38.3	$<10^{-5}$
CZ	succession	flies (larvae)	all	3 / 744	9.3	$<10^{-4}$
CZ	succession	flies (larvae)	saprophages (all)	3 / 744	9.1	$<10^{-3}$
CZ	succession	flies (larvae)	omnivores	3 / 744	26.1	$<10^{-5}$

data = from which data set the trend has been calculated (CZ = Czech Republic data, UK = United Kingdom data, combined = CZ+UK), temporal gradient = which temporal gradient was used as the environmental variable (season or succession), order = the model was fitted for either beetles or flies, functional group = for which functional group the model was fitted (all = all beetles or flies, saprophages = both adults and larvae coprophagous (dwellers = larvae develop in the dung pats, relocators = larvae develop outside of the dung pat), omnivores = trophic switch between adults and larvae (usually adult saprophage, larva predator), predator = both adult and larvae predatory), df = degrees of freedom (1 dung pat = 1 observation) of that particular GLM in format: model used df / residual df (model df 2 = linear curve, model df 3 = quadratic curve).

doi:10.1371/journal.pone.0170426.t002

The pattern of seasonal displacement between beetles and flies was also seen at both individual sites (Fig 1C, 1D and 1E), with beetles being abundant in spring–autumn while flies were most abundant in summer in CZ, and with beetles being the most abundant in the earlier part of the season (spring), while flies were the most abundant in the later part of the season (mostly late summer) in the UK.

Seasonal-temporal patterns of beetle and fly functional groups

The majority of ecological groups displayed a significant trend along the seasonal gradient in the combined CZ+UK data and also in site-specific datasets (Table 2).

The saprophagous beetles in the combined data were clearly numerically separated seasonally from the saprophagous flies (Fig 2A), being most abundant during spring and autumn; while saprophagous flies were most abundant during the summer. The same pattern was also retrieved at both sites, especially at the CZ site (Fig 2C). The saprophagous beetles and flies were also separated in season at the UK site, but saprophagous beetles, although on the verge

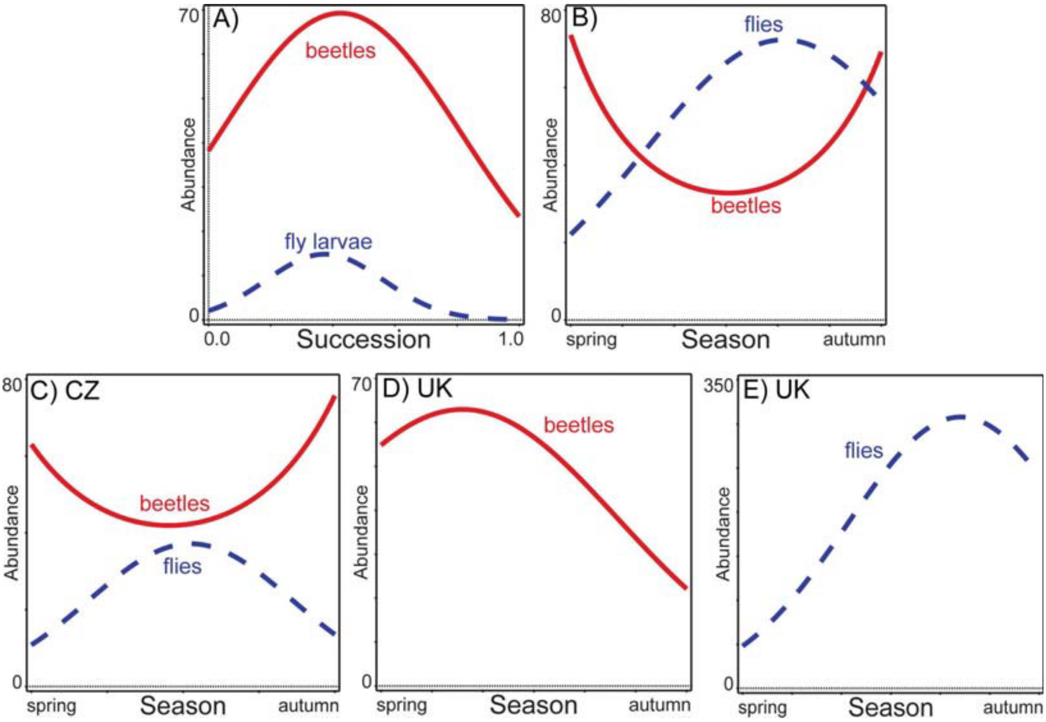

Fig 1. Trends of dung-inhabiting beetle and fly abundances during succession (Czech Republic) and season (both sites). A) successional trends of dung-inhabiting beetles (solid red line) and fly larvae (dashed blue line) (data from the Czech Republic), B) seasonal trends of dung-inhabiting beetles (solid red line) and flies (dashed blue line) (data from both sites), C) seasonal trends of dung-inhabiting beetles (solid red line) and flies (data from the Czech Republic), D) seasonal trends of dung-inhabiting beetles (data from the United Kingdom), E) seasonal trends of dung-inhabiting flies (data from the United Kingdom),

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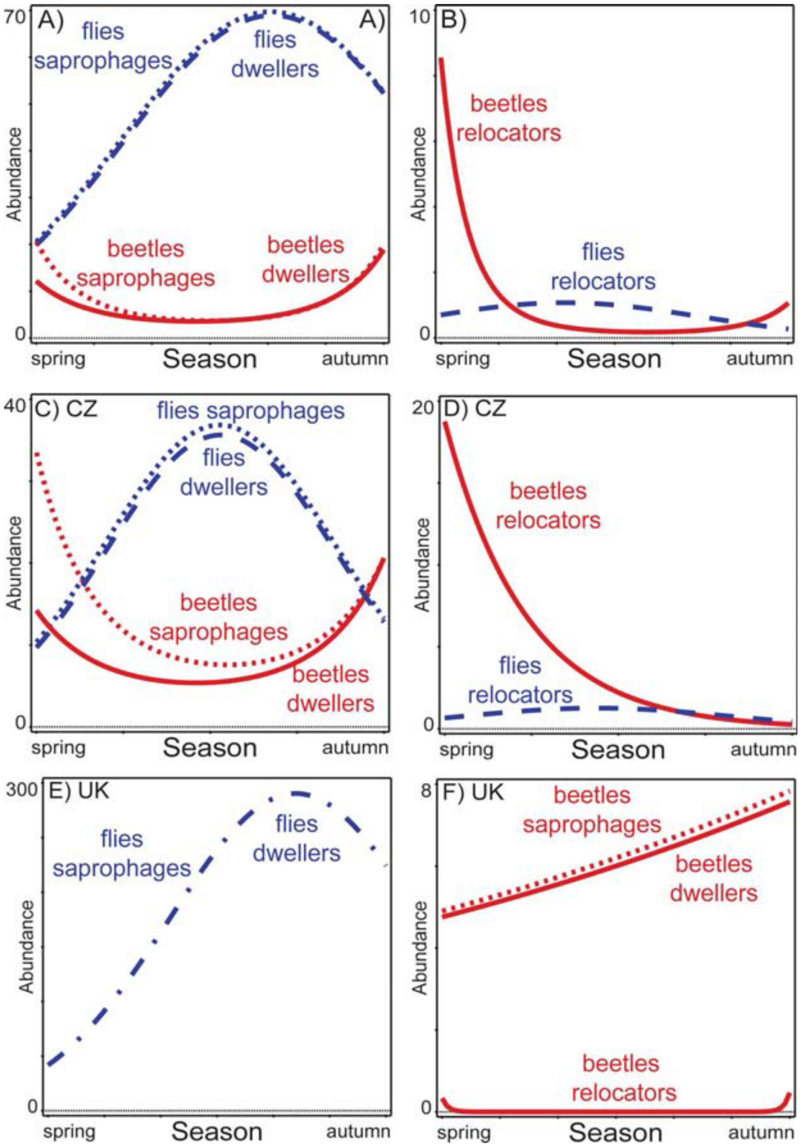

Fig 2. Seasonal trends of saprophagous dung-inhabiting beetles, flies and their guilds in the combined data and data from both sites. Seasonal trends are presented for the combined data (A, B), data from the Czech Republic (C, D) and data from the United Kingdom (E, F). The fly patterns are represented by blue lines, the beetle patterns are represented by red lines. Dwellers = species whose larva develop in the dung pat, relocators = species whose larva develop outside of the dung pat.

doi:10.1371/journal.pone.0170426.g002

of significance ($P = 0.05$), increased their numbers in the later part of season when fly numbers were decreasing (Fig 2E).

The seasonal patterns of all saprophagous beetles and flies for both the combined and site-specific datasets were mimicked by the responses of the saprophagous beetle and fly dwellers guilds, as the majority of saprophagous beetles and flies were dwellers (Fig 2A, 2C and 2E).

The second saprophagous guild of relocators also displayed seasonal separation between beetle relocators, dominating spring and partly autumn, and fly relocators, dominating summer (Fig 2B) in combined data. A similar separation among beetle and fly relocators was also retrieved in the site-specific data (Fig 2D and 2F).

The omnivorous beetles did not display a significant seasonal trend in the combined data ($F = 2.1$, $P = 0.13$), while the number of omnivorous flies increased throughout the season (Fig 3A). In contrast, the omnivorous beetles and flies were seasonally separated at individual sites (Fig 3C, 3D and 3E).

Despite that the fact that predatory flies did not reach abundances comparable with predatory beetles (107 vs. 7385, see Table 1), they did display a clear separation from the predatory beetles by their short occurrence in early summer, while the numbers of predatory beetles were almost the lowest in the combined data (Fig 3B). A similar pattern also applied for both individual sites, where predatory flies displayed a peak of abundance while the predatory beetles had their minimum abundance in CZ (Fig 3C and 3D), or when the number of beetle predators were declining in the UK (Fig 3F).

Seasonal-temporal patterns of beetle and fly species

The species assemblages were significantly structured by season in both the CZ data ($F = 12.6$, $P = 0.001$, all axes explain 6.4% of variability in species data) and UK data ($F = 17.3$, $P = 0.001$, all axes explain 30.9% of variability in species data). In general, species seasonal patterns highly support the results of GLMs. This is most recognizable in the CZ beetle and fly saprophages, where beetle species almost exclusively preferred spring and late-summer/autumn seasons, while fly species greatly preferred early and late summer seasons (Fig 4A). In CZ omnivorous species, beetles reached their optima from the early summer to autumn with most species preferring the late summer/autumn season, while fly species were almost equally distributed between spring and autumn seasons (Fig 4B). Two thirds of CZ beetle predatory species reached their optima in either spring or late summer/autumn seasons with one third occurring between early and late summer (Fig 4C). On the other hand, CZ fly predators occurred almost exclusively in early summer or between late spring and early summer (Fig 4C). In the UK data, there were interchanges between late spring occurring beetle predators and early summer occurring fly predators, and again late spring occurring beetle omnivores and late summer/autumn occurring fly omnivores (Fig 4D). In saprophages, the majority of UK fly saprophages were associated with early/late summer, while some beetle species also occurred in the summer seasons, but the most abundant species had their optima in late summer/autumn seasons (Fig 4D).

Successional-temporal patterns of beetle and fly functional groups

The optima of both saprophagous beetles and saprophagous fly larvae almost overlapped along the successional gradient (Fig 5A). At the individual guild level, the larvae of saprophagous flies, represented exclusively by fly larvae dwellers, had their peak abundance slightly after the highest abundance of beetle relocators and almost together with the maximum abundance of beetle dwellers (Fig 5B). The larvae of omnivorous beetles had their maximum just slightly after the peak in abundance of omnivorous fly larvae (Fig 5C) (Table 1).

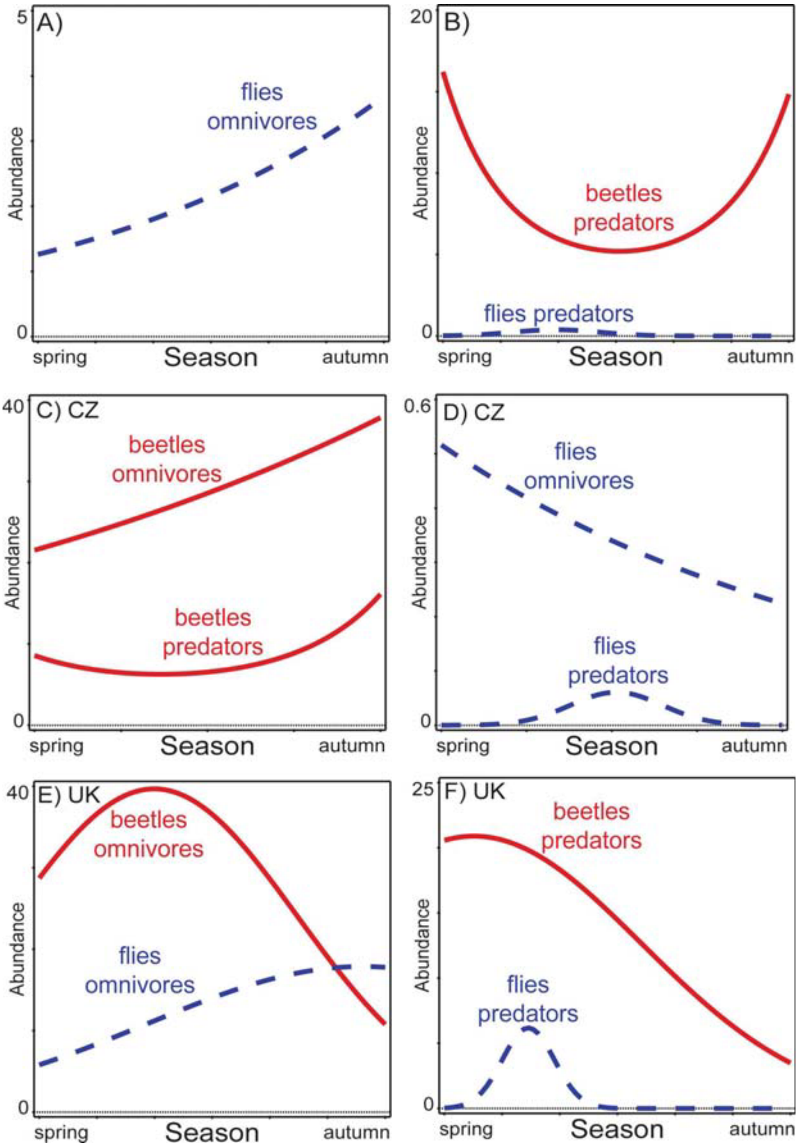

Fig 3. Seasonal trends of omnivorous and predatory dung-inhabiting beetles and flies in the combined data and the data from both sites. Seasonal trends are presented for the combined data (A, B), data from Czech Republic (C, D) and data from United Kingdom (E, F). The fly patterns are represented by blue lines, the beetle patterns are represented by red lines. Omnivores = trophic shift between adult and larva (adult usually saprophagous, larva predatory).

doi:10.1371/journal.pone.0170426.g003

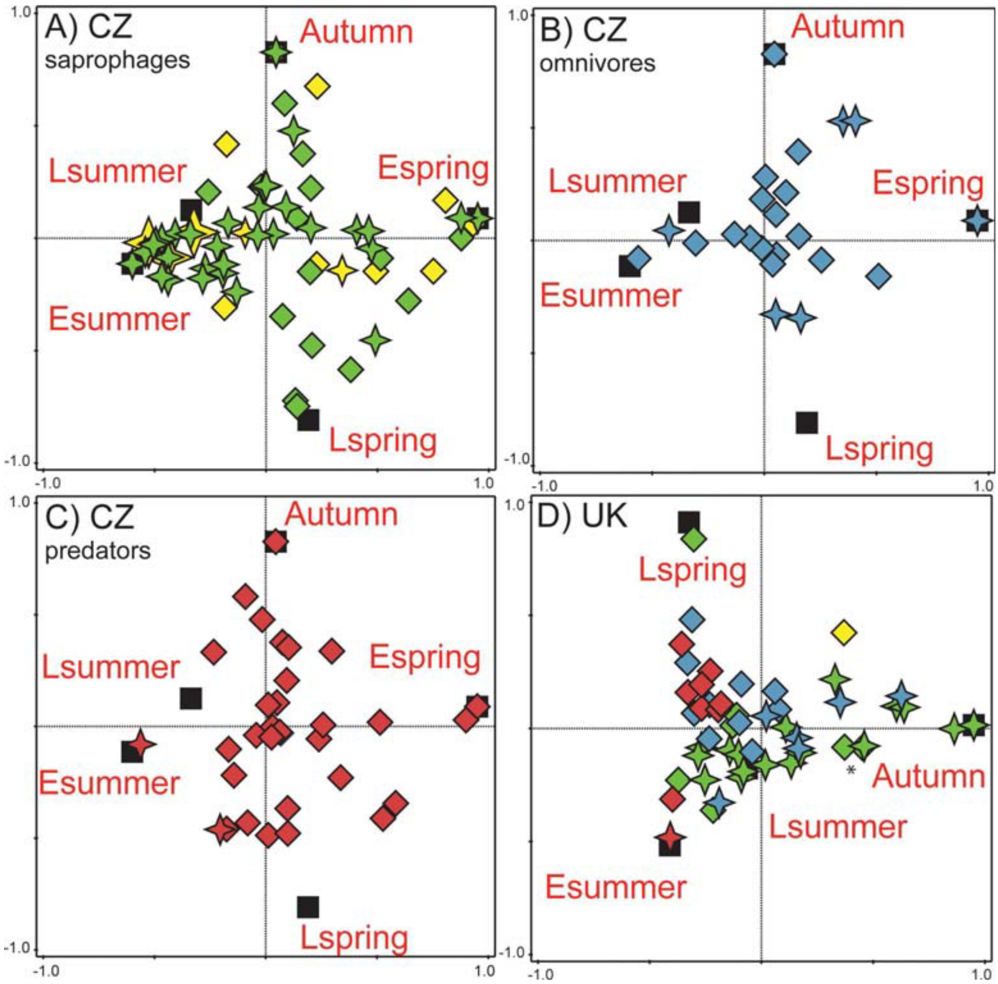

Fig 4. DCCA ordination diagrams of species seasonal segregation at both sites. Panels A, B and C represents the seasonal segregation of CZ species, all originating from one analyses ($F = 12.6$, $P = 0.001$). Those panels were created by including just one functional group for better visualization of results (A = saprophages (both adults and larvae coprophagous), B = omnivores (trophic switch between adults and larvae (usually adult saprophage, larva predator)), C = predators (= both adult and larvae predatory). Panel D represents the overall results of seasonal segregation in UK species ($F = 17.3$, $P = 0.001$). black squares = centroids of individual seasons (Espring = early spring (April–first half of May), Lspring = late spring (second half of May–June), Esummer = early summer (July), Lsummer = late summer (August–early days of September), Autumn = second half of September–October), diamonds = beetle species, stars = fly species, yellow symbols = relocators (larvae develop outside of the dung pat), green symbols = dwellers (larvae develop in the dung pats), blue symbols = omnivores, red symbols = predators, an asterisk (*) = the most abundant saprophage species in the UK data.

doi:10.1371/journal.pone.0170426.g004

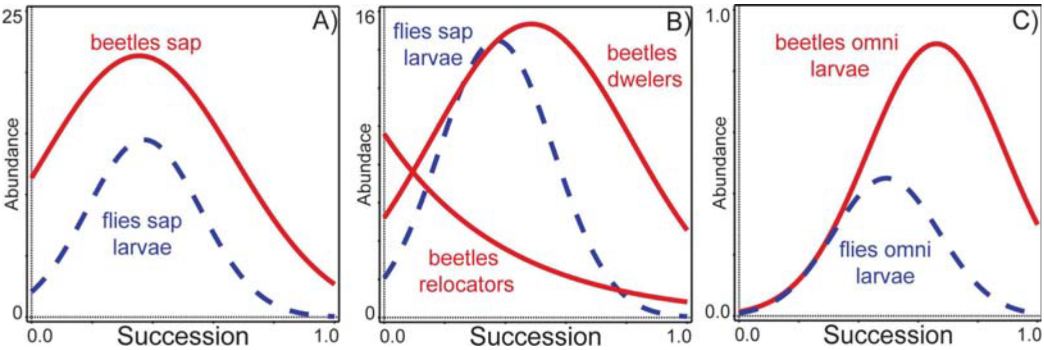

Fig 5. Successional trends in dung-inhabiting beetles and flies. A) beetle (solid red line) and fly larvae (dashed blue line) saprophages (sap); B) beetle relocators and dwellers (solid red lines) and fly larvae (dashed blue line) saprophages (sap); C) beetle omnivorous larvae (omni: solid red line) and fly omnivorous larvae (omni: dashed blue line).

doi:10.1371/journal.pone.0170426.g005

Discussion

Herein we have shown that the coprophilous beetles and flies, as the main components of dung-inhabiting communities, display relatively robust temporal separation at both order and species level. In both the combined data from our sites and at individual sites, the periods of peak abundance of the coprophagous and predatory flies and beetles with similar ecology appear to avoid each other during the season (i.e. niche differentiation). The omnivorous beetles and flies were not temporally separated in the combined data, but they displayed a solid seasonal separation at individual sites at both order and species level. In contrast, the potential competitors from among the coprophagous and omnivorous functional groups co-occurred along the successional gradient (i.e. habitat filtering).

Biotic and abiotic interpretations of temporal segregations

The seasonal separation between all beetles and flies, and especially among representatives of ecological groups could have two potential explanations. The temporal dynamics of the coprophilous insect temperate community reflect either the biotic interactions of its species, i.e. competition or predation (niche differentiation) [5, 7]; or they reflect the patterns of species adaptation to certain environmental conditions (habitat filtering) [2, 4].

The seasonal separation among both beetles and flies and their respective functional groups should indicate niche differentiation [3, 6]. The present temperate community of coprophilous insects should therefore be formed as a result of recent or historical competition, or even possibly intra-guild predation [54].

The asymmetrical competition between saprophagous beetles and flies is well-known, as large dung relocating beetles can quickly deplete dung for the dung dwelling fly larvae [55]. This competition is, however, restricted to regions with a dominant presence of such dung relocating beetles, i.e. the tropical, sub-tropical and Mediterranean regions [e.g. 56]. In contrast, the temperate beetle communities are comprised almost exclusively of beetles whose larvae also develop in the dung pats (dwellers) [49]. The relationship of those dwelling beetles and flies are, however, rather complex. The dwelling beetles could negatively affect fly larvae survival, by destroying their eggs [57]. On the other hand, beetles tend to avoid oviposition in the presence of high abundances of fly larvae [58]. Finally, if the fly larvae are excluded from dung pats, the saprophagous beetle adults and larvae abundances are affected negatively [59]. We therefore suggest that, given the current evidence, the temporal separation of temperate saprophagous beetles and flies cannot be satisfactorily explained by recent competition.

Historical competition between beetles and flies could be a viable explanation. Many species of the Czech and British coprophilous fauna probably dispersed from their Mediterranean glacial refugia [60–62] since they have ranges spanning from the Mediterranean region northward [63, 64]. In that case, the coprophilous insects could still follow the temporal patterns established in the Mediterranean region. Here the saprophagous beetles, especially the competitively dominant large dung relocating species also occur primarily in spring and partly in autumn [38, 56, 65]. Flies on the other hand should be most abundant during summer and early autumn [55, 66, 67].

Biotic interactions could also play an important role in facilitating the temporal patterns of omnivores and predators. In those two trophic groups, potential intra-guild predation could play the major role, since it can affect the predated species behavior or habitat choice [68–70]. In both such groups, the beetles presumably play the dominant role over the flies in the seasonal separation. In omnivores, the fly larvae are very similar to the larvae of other flies, having only different modification of their mouth hooks [71]. In contrast, the larvae of beetles have hard-biting mouthparts and are generally ferocious predators of fly larvae in general [46, 72]. Despite the fact that the larvae of predatory flies can kill the larvae of bark beetles [73], they probably cannot compete against the mobile and voracious larvae of predatory beetles, nor their adults.

Despite the fact that seasonal separation of coprophilous beetles and flies indicates niche differentiation, there is more speculation than actual evidence for real competitive segregation.

Habitat filtering on the other hand might also be a valid explanation for the beetle and fly seasonal patterns. Contrary to niche differentiation, habitat filtering aggregates species with similar traits, mostly because of their tolerances to the abiotic environmental factors–which should change along the seasonal gradient in our study [2, 4]. The most obvious, seasonally dependent, environmental factor in temperate environments is temperature.

The relations between beetles, flies and the ambient temperature have not been studied extensively; however, they could provide a simple interpretation for the seasonal displacement between those groups in temperate environments. Temperate dwelling saprophagous beetles should be susceptible to higher temperatures (>30°C), especially in larval stages [74, 75]. On the other hand, such beetles can be active in relatively low temperatures (5–10°C) [74]. The same principles probably apply also to omnivorous and predatory beetles, as well as to fly omnivores [41, 76]. In contrast, the majority of saprophagous flies need higher temperatures to become active (>10°C), but they are more resistant to higher temperatures in general [41]. In fact, higher temperatures enable their larvae to finish their development more quickly, avoiding potential predation [41]. In addition, the temperature-based separation of beetles and flies also applies in the Mediterranean region, since high temperatures could be lethal to dung relocating beetles if they do not possess any heat-regulating ability [31]. Finally, the seasonality based upon temperature tolerance would be a very simple explanation as to why the European species display an identical seasonal pattern in artificially-formed communities in North America in which the majority of species are immigrants from Europe [42, 77].

In contrast to the seasonal patterns, there were no temporal separations among the species utilizing the resource in the same way during the succession of coprophilous beetles and flies. Successional patterns in this community indicate *a priori* habitat filtering, in which species with similar ecology tend to aggregate in the succession, probably along with favorable chemical or moisture conditions [30, 37].

Temporal trends in dung and other systems

We find environmentally-based filtering to be the most parsimonious explanation for the assembly of the temperate coprophilous insect community, as it is probably driving both the successional and seasonal gradients. Our findings agree with the prediction [13–17] that environmental filtering is the main community assembly mechanism, applying also in dung and in other insect communities inhabiting other ephemeral habitats [78, 79].

On the other hand, the niche-based separations among coprophilous insects and insects inhabiting similar ephemeral habitats [23, 80] could take place on the smaller scale of individual sites. For example, even if coprophagous flies and beetles are seasonally separated, the coprophagous representatives of both groups are temporally aggregated and co-exist probably in niche-based separation [34], i.e. macro- (e.g. forest vs. open field) or microhabitat (dung type, dung pat size) preferences.

Site differences, study limitations and future suggestions

Both the combined and site-specific data depicted the main result as seasonal separation between coprophilous beetles and flies, and their respective functional groups. The site-specific data differed sometimes slightly in the shapes of the seasonal patterns, e.g. omnivorous beetles were mostly sampled throughout the spring and partly in the summer in UK, while they were the most abundant in the autumn in CZ. Such differences could stem from the different sampling methods used at both sites, since the CZ data contain the community actually present in the dung pat, whereas the UK data present who is leaving the pat, including reared individuals. Taking omnivorous beetles as an example, they should be therefore most abundant in autumn (according to the CZ data), but reproduce more in spring (according to the UK data).

Our hypothesis that habitat filtering is the main assembly rule in coprophilous insects, and probably in other communities inhabiting ephemeral habitats, needs of course rigorous testing in further studies. We therefore suggest that the main pathways for future studies should include: 1) dung pat physical (e.g. moisture development) and chemical (e.g. dung volatiles, and other dung chemistry) attributes during succession (paired with testing the species' affinity or resistance to them); 2) species' temperature tolerances/affinities; or 3) detailed studies on the competitive and trophic interaction between beetle and fly species.

Supporting Information

S1 Table. Ecological classification of dung-inhabiting beetles and flies used in this study. Fun. group = functional group (saprophages = both adult and larvae saprophagous, omnivores = trophic shift between adult and larva (adult usually saprophagous, larva predatory), predators = both adult and larva predatory), dwellers = species whose larva develop in the dung pat, relocators = species whose larva develop outside of the dung pat, model representative = an example of a species belonging to that exact functional group/taxonomic group and was present in our sampling. If a taxonomic group was not identified beyond the genus level, the model representative is genus spp., if identification was not possible beyond the family level, no representative is given.
(DOC)

Acknowledgments

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 the CZ part of this study on their pasture. We are equally grateful to Mr John Keedwell for permission to carry out the UK part of the study on his farm. We also thank Vojtech Novotny, Martin Volf and Tomas Zitek for comments on an earlier version of the manuscript.

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Investigation: FXJS CL.

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Resources: MK RW.

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Validation: FXJS STS.

Visualization: FXJS.

Writing – original draft: FXJS.

Writing – review & editing: FXJS STS RW MK.

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Supporting Information

Table S1: Ecological classification of dung-inhabiting beetles and flies used in this study.

Fun. group = functional group (saprophages = both adult and larvae saprophagous, omnivores = trophic shift between adult and larva (adult usually saprophagous, larva predatory), predators = both adult and larva predatory), dwellers = species whose larva develop in the dung pat, relocators = species whose larva develop outside of the dung pat, model representative = an example of species that belongs into that exact functional group/taxonomic group and was presented in our sampling. If a taxonomic group was not identified behind genus level, the model representative is genus spp., if identification was not possible behind family level, no representative is given.

Order	**Family**	**Sub-family**	**Fun. group**	**guild**	**model representative**
beetles	Carabidae	-	predators	-	*Amara aenea* (De Geer, 1774)
beetles	Geotrupidae	-	saprophages	relocators	*Geotrupes spiniger* (Marsham, 1802)
beetles	Histeridae	-	predators	-	*Hister unicolor* Linnaeus, 1758
beetles	Hydrophilidae	-	omnivores	-	*Sphaeridium bipustulatum* Fabricius, 1781
beetles	Scarabaeidae	Aphodiinae	saprophages	relocators	*Aphodius erraticus* (Linnaeus, 1758)
		Aphodiinae	saprophages	dwellers	*Aphodius ater* (De Geer, 1774)
		Scarabaeinae	saprophages	relocators	*Onthophagus coenobita* (Herbst, 1783)
beetles	Staphylinidae	Oxytelinae	saprophages	dwellers	*Anotylus rugosus* (Fabricius, 1775)

beetles	Staphylinidae	Proteininae	saprophages	dwellers	*Megarthrus depressus* (Paykull, 1789)
		other subfamilies	predators	-	*Philonthus cruentatus* (Gmelin, 1790)
flies	Anthomyiidae	-	saprophages	-	-
flies	Calliphoridae	-	saprophages	relocators	*Lucilia casear* (Linnaeus, 1758)
flies	Carnidae	-	saprophages	dwellers	*Meoneura* spp.
flies	Chironomidae	-	saprophages	dwellers	-
flies	Chloropidae	-	saprophages	relocators	-
flies	Dolichopodidae	-	predators	-	-
flies	Empididae	-	predators	-	*Empis* spp.
flies	Fanniidae	-	saprophages	dwellers	*Fannia* spp.
flies	Hybotidae	-	predators	-	*Drapetis* spp.
flies	Limoniidae	-	saprophages	dwellers	*Rhipidia maculata* Meigen, 1818
flies	Milichiidae	-	saprophages	dwellers	*Madiza glabra* Fallén, 1820
flies	Muscidae	Muscinae	omnivores	-	*Mesembrina meridiana* (Linnaeus, 1758)
		Muscinae	saprophages	dwellers	*Musca autumnalis* De Geer, 1776
		Mydaeinae	omnivores	-	*Hebecnema vespertina* (Fallén, 1823)
		Mydaeinae	saprophages	relocators	*Myospila meditabunda* (Fabricius, 1781)
flies	Sarcophagidae	-	saprophages	dwellers	*Ravinia pernix* (Harris, 1780)
flies	Scathophagidae	-	omnivores	-	*Scatophaga stercoraria* (Linnaeus, 1758)
flies	Scatopsidae	-	saprophages	dwellers	-
flies	Sciaridae	-	saprophages	dwellers	-
flies	Sepsidae	-	saprophages	dwellers	*Saltella sphondylii* (Schrank, 1803)

flies	Sphaeroceridae	-	saprophages	dwellers	*Lotophila atra* (Meigen, 1830)
flies	Stratiomyidae	-	saprophages	dwellers	*Sargus flavipes* Meigen, 1822
flies	Syrphidae	-	saprophages	dwellers	-

CHAPTER III

Frantisek Xaver Jiri Sladecek, Tomas Zitek, Martin Konvicka, Simon Tristram Segar. *How do the temporal trends of dung-inhabiting predators affect their coexistence?* (manuscript).

How do the temporal trends of dung-inhabiting predators affect their coexistence?

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Abstract

The assembly of natural communities and the coexistence of species within these communities is a major issue in community ecology. Community assembly is mostly influenced by species’ adaptability to environmental conditions (habitat filtering) or by the avoidance of competition. Such mechanisms have been almost exclusively studied in primary producers or consumers, and there has been a considerable paucity of studies focusing on the assembly, and thus mechanisms behind creation and coexistence, of predatory communities. We therefore present a study focusing on the coexistence and assembly of a species rich community of dung-inhabiting predators. We use field data in combination with morphology to assign trophic links before creating a food web of dung-inhabiting insects. Using this we infer negative interactions, competition and intraguild predation, as these interactions could threaten the long term co-existence of dung-inhabiting predators. We analyzed the potential for predatory species' temporal trends across a gradient of succession and seasonality, to mediate negative interactions through either environmental filtering or niche differentiation. The ratio of predators to prey increased throughout succession, although it remained constant among seasons. There was decreasing trend in predator size along the successional gradient, while similarly sized predators were evenly distributed across seasons. The succession of dung-inhabiting predators therefore displayed clear potential for environmental filtering. In contrast, seasonality could promote coexistence of species co-occurring in succession, as their combination seems to drastically lower the potential for negative interactions.

Keywords: community assembly, competition, dung, habitat filtering, intraguild predation, predators

Introduction

Understanding species coexistence is a crucial for understanding the composition of natural communities. Mechanisms preserving species rich communities from collapsing into single species dominions (Gause, 1934) are therefore rightly among the most popular topics in community ecology.

Natural communities are usually considered to be assembled and maintained through species adapting to the environmental conditions (habitat filtering) (Keddy, 1992; Kraft et al., 2015) or avoiding negative interactions such as competition and predation (niche differentiation) (MacArthur & Levins, 1967; Schoener, 1974). Habitat filtering thus leads to the coexistence of species with similar traits related to environmental tolerance (Maire et al., 2012), e.g. temperature tolerance (Sladecek et al., 2017a). Niche differentiation leads to communities with species possessing different traits related to resource acquisition (Maire et al., 2012), e.g. temporal shifts in resource utilization (de Camargo et al., 2016) or spatial avoidance (Opatovsky et al., 2016). Although there is no consensus about which of these mechanisms dominates, habitat filtering seems to apply at larger spatial scale, i.e. separation among distantly related phylogenetic clades, while niche differentiation seems to promote species coexistence within those clades (Adams & Thibault, 2006; Arellano et al., 2016; de Camargo et al., 2016; Wiescher et al., 2012). Both of these mechanisms have been extensively studied in plant and herbivorous/saprophagous animal communities.

In contrast, studies focusing on the assembly of predatory communities, i.e. predators *s.stricto*, parasitoids and parasites, etc., are rather scarce or limited in scope. Most studies have focused on predators from the perspective of their prey, e.g. herbivorous/saprophagous animals. This includes the role of predators in the assembly of their prey communities (Giam & Olden, 2016; Paine, 1966) or disassembly of their prey communities as part of biological control (Horenstein & Salvo, 2012; Walsh & Cordo, 1997). Studies involving the coexistence of predators themselves have been primarily limited to pairs of predatory species or small subsets of much larger communities (Bischof et al., 2014; Droge et al., 2017; Hawes et al., 2013; Wereszczuk & Zalewski, 2015). In this work, we will therefore focus on community assembly of predators communities using solely the relations among predatory species.

Niche differentiation is therefore considered to play a major role in the coexistence of predatory species (e.g. Droge et al., 2017; Torretta et al., 2016), although there is evidence that a combination of both niche differentiation and habitat filtering occurs in some systems (Wereszczuk & Zalewski, 2015). This coexistence is threatened by indirect or direct interactions among predators. The indirect interactions include competition via the lowering of shared prey abundance (exploitation competition) (White et al., 2006). The direct interactions include physical contact (fighting or killing) among predators, (interference competition)(Hawes et al., 2013) or direct predation (intraguild predation, IGP) (Holt & Huxel, 2007). Of these interactions, the direct ones are probably the most detrimental for predatory species (Arim & Marquet, 2004; Gagnon et al., 2011; Raso et al., 2014). There is plenty of evidence to suggest that species pairs or small subsets of communities, be it vertebrates or invertebrates, avoid potential competition/IGP via changes in their behavioural patterns (Koivisto et al., 2016; Mueller et al., 2016), host-specificity (e.g. Hrcek et al., 2013), or through spatio-temporal segregations (Bischof et al., 2014; Droge et al., 2017; Opatovsky et al., 2016). While all such observations come from relatively stable communities, there is however, a notable lack of studies that focus on coexistence between predators inhabiting energy rich, yet temporally unstable ephemeral habitats like carrion or animal dung.

The coexistence of dung-inhabiting predators has always been a neglected topic, especially when compared to the coexistence of dung-inhabiting saprophages (Finn et al., 1998; Hanski, 1980a; Holter, 1982; Sladecek et al., 2013). This is despite the fact that predatory species constitutes over half of temperate dung- insect species richness (Hanski & Koskela, 1977; Sladecek et al., 2013), and also form a significant part of tropical communities (Guimaraes & Mendes, 1998; Walsh & Posse, 2003). Dung-inhabiting predators are clearly subject to direct interactions, be it IGP or interference competition, as an increase in predator abundance does not result in an increased overall predation rate (Fincher, 1995; Roth, 1982). in the role of indirect interactions, e.g. exploitative competition, less clear, due to abundance of their prey (Valiela, 1974). Although some dung-inhabiting predators are genuinely voracious (Valiela, 1969). There is, however, some information scattered in the ecological literature, e.g. the documented ability of predators as bio-control agents. The coexistence of dung-inhabiting predatory species, at the community scale, is considered to be maintained by spatio-temporal segregations (Hanski & Koskela, 1979; Sladecek et al., 2013), as dung-inhabiting predatory species (at least in temperate regions) display fixed successional and seasonal patterns (Koskela, 1972; Sladecek et al., 2013; Wingo et al., 1974). Despite this strong support for niche differentiation, it has also been suggested that habitat filtering might play a role in the seasonal separation of beetle and dipteran predators (Sladecek et al., 2017a), as well as in both the successional and seasonal separation of beetle predators (Hanski, 1980b; Sladecek et al., 2013). A negative relationship between size and occurrence (across successionary or seasonal gradients) offers a mechanism here. Apart from these patterns, the co-existence of dung insects has not been further studied (e.g. there has been no consideration of potential competition of IGP to date).

The main focus of this study is to investigate the potential mechanisms shaping the coexistence of predatory dung insects. To investigate this, we inferred their potential negative indirect (as "competition" for same resource) and direct interactions (as "IGPIC" for IntraGuild Predation and Interference Competition). This was done using a combination of field data and trophic links based on the size ratio of predators to prey, assuming that predator eats smaller prey/predator (Kajita et al., 2014; Woodward et al., 2005). Using these negative interactions, we attempted to assess whether the predators' temporal trends, succession and seasonality, could be a result of their niche differentiation or environmental filtering (Keddy, 1992; Kraft et al., 2015; Silvertown, 2004). The potential for species' succession and seasonality to lower or eliminate negative interactions among dung-inhabiting predators is presented at two community levels; a) predatory guilds (conglomerates of similar sized species) and b) individual predatory species.

Materials and Methods

Study site

This 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 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., 2017b)

Data sampling

Three sampling seasons were carried out in 2011 (spring: 18 April – 3 May; high summer: 12–27 July; and late summer/early autumn: 22 August – 6 September), covering the main periods of dung-inhabiting insect activity (Sladecek et al., 2013; Sladecek et al., 2017b).

Insect communities were sampled from artificially created dung pats of 1.5 l volume. Fresh and un-colonized dung was gathered from a barn with permanently stalled cows. The dung pats were then artificially created at the study site. The artificially created dung pats contain the same insect communities as naturally dropped ones (Barth et al., 1994).

In each sampling season, we sampled insect communities from dung pats exposed for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 days. Very fresh dung (up to 1 day old) and very old dung (from day 12 onward) was not included since no species living within dung reach their peaks of abundance in very fresh or very old dung pats (Sladecek et al., 2013). Each successional age of dung was replicated four times per season. These four replications in each season (one replication = line of 11 dung pats covering the whole successional gradient) were exposed one by one during four consecutive days to minimize the effects of weather variability on insects activity. The position of pats designated to individual successional ages was randomized within each replicate line. The sampling thus comprised 44 dung pats per sampling season and 132 dung pats in total through three sampling seasons. The dung pats presented in this study were used as un-manipulated controls in another study (Sladecek et al. in prep.) and to compare the temporal patterns of fly larvae with beetle patterns in our other study (Sladecek et al., 2017a).

Insects were extracted by floating the dung pat and a small portion of underlying soil in a bucket of water. The floated substrate was then hand-sorted to assure that all individuals were sampled. The insects were preserved in 96% ethanol and taken to the laboratory for identification. The community inhabiting one dung pats represents one sample in all further analyses.

Prey spectra assignment rules in our study

To assess the potential competition and IGPIC between predators we first established the total prey/IGPIC prey spectra for each predator or predatory guild (who can eat whom). We then revised the links according to the temporal patterns of predator and prey species' (one can eat another if and only if they co-occur in the same age dung pat or in the same season).

The total prey spectra was based primarily on the relationship between predator and prey sizes, as size is probably the main dimension affecting prey selection (Kajita et al., 2014; Woodward et al., 2005). We made the *a priori* assumption that adult beetle (predators) can prey upon similarly sized (saprophages) or smaller adult beetles (saprophages, predators), for which there is ample evidence (mostly from: Hammer, 1941; Laurence, 1954; Mohr, 1943; Valiela, 1969, 1974; Walsh & Cordo, 1997; Wu et al., 2011; Wu et al., 2014). In addition, we presume that all adult beetle predators are capable of preying upon both parasitoids and larvae (both saprophage and predatory), behaviour for which there is also an abundance evidence (Fincher, 1995; Hammer, 1941; Laurence, 1954; Roth, 1982; Valiela, 1969). Finally, we assume that predatory larvae and parasitoids prey only on beetle and fly larvae, since carnivorous larvae are too sluggish to attack agile beetles, and parasitoids will obviously just parasitise larvae.

Finally, we wanted to focus on predators relations rather than prey relations, and consider predators to feed primarily on other living insects rather than on dung. We therefore omit dung as the primary resource from further trophic relations.

Statistical analyses

Guilds patterns

First, we defined the trophic guilds. Guilds of adult beetles (both predatory and prey) were defined by Ward's hierarchical clustering along their size in R 2.15.2 (Team, 2012). Optimal number of guilds/clusters was identified by K-means clustering. The adult size of individual species was represented by its average dry weight. One of us (Zitek) weighted 20 adult individuals of each dung-inhabiting beetle species present at our study site and calculated the mean dry weight for each species (Zitek et al. in preparation) (Table S1). As dry weight is not a completely representative trait for larvae and parasitoids we *a priori* classified the prey larval individuals according to their obvious live size and ecological differences as "large" (Calyptrate flies; e.g. Muscidae) and "small"

(Acalyptrate flies, e.g. Sepsidae) saprophage larvae (Hammer, 1941; Laurence, 1954). "Predatory larvae" (carnivorous larvae of Muscidae, Hydrophilidae) and "parasitoids" (various Hymenoptera families) formed their own guilds as well (Hammer, 1941; Mohr, 1943).

Next, we created a table of all possible predatory guild combinations. For each pair (e.g. predatory larvae and parasitoids) we identified potential IGPIC (using prey spectra assignment rules) and competitive relations. The competitive relations were based upon shared predatory links in each guilds bipartite food web. The food web was created by analyzing the responses of each predatory guild to all recovered prey guilds. This was done using the Generalized linear models with mixed effects (GLMM) with a Poisson distribution of errors in the R package "lme4" (Bates et al., 2013). We fitted a model for each individual predatory guild + prey guilds (as predictors), while dung age, season and affinity to replicative line were the random effects. A predatory guild received a trophic link to a prey guild if: a) the predator guild was significantly positively affected by this prey guild; and b) if this prey guild is suitable for this predatory guild (using the prey spectra assignment rules). The web was visualized using the R package "bipartite" (Dormann et al., 2008). This table thus represented the basic hypothesis for the potential prevelance of IGPIC and competition among dung-inhabiting predators.

Finally, we analyzed the temporal trends of each guild, and which of them (succession, seasonality), could prevent competition and IGPIC among dung-inhabiting predators. We fitted two generalized linear models (GLM) for each guild, one with season and one with succession as environmental variable, this allowed us to compare temporal avoidance and co-occurrences between guilds at both seasonal and successional scales. GLMs were fitted as quadratic, to identify temporal optima, and with quasipoisson distribution of errors, to eliminate overdispersion. Such GLMs were fitted as species response curves in CANOCO for Windows 5 (Ter Braak & Smilauer, 2012). By comparing the temporal trends for the guilds we estimated if IGPIC or competition between guilds could be: a) avoided (both guilds have significant yet different trends along the temporal gradient); b) not avoided (guilds have significant and similar trends along the temporal gradient); or c) unresolved, due to one or both guilds not having a significant trend along the temporal gradient.

Species level patterns

To analyze the influence of species level temporal trends on competition and IGPIC, we constructed the potential overall food web of dung-inhabiting insects. The overall food web was constructed based upon both prey spectra assignment rules, and predator-prey temporal co-occurrence, because we assume that predators prey solely or predominantly upon species with which they co-occur in succession and season. To quantify this co-occurrence, we computed Spearman's correlation coefficient for each predator and prey species using the "rcorr" function in ‘Hmisc’ package in R 2.15.2 (Harrel & Dupont, 2014). Predators therefore received a trophic link to a prey species if the prey was in its prey spectrum providing that both were significantly and positively correlated in our data. The final web was visualized twice and basic network statistics were computed in package ‘bipartite’ in R (Dormann et al., 2009; Dormann et al., 2008); one with species sorted by their successional and the other with species sorted by their seasonal optima. Species successional and seasonal optima were retrieved from two Canonical Correspondence Analyses (CCA), one with succession and second with season as environmental variable, computed in CANOCO 5 for Windows (Ter Braak & Smilauer, 2012). CCA is a multivariate technique suitable for datasets with a unimodal species response to the gradient, i.e. displaying optima (Leps & Smilauer, 2003). Both CCAs were carried out with the whole insect community, excluding species appearing in only one sample, and the response variables and succession or season as environmental variable. The affinity to a replicative line and opposite temporal variable (for analysis with succession, season is the opposite) were treated as covariables. Significance of the CCAs was tested using Monte Carlo permutation tests (999 permutations).

Next, we identified groups of predatory species that co-occur and thus could interact together, sharing the same prey in the species food web, or involved in IGPIC (using the species membership in guilds as predetermination for IGPIC relations). We clustered predatory species based upon their successional optima. The successional gradient was chosen because we have more detailed data (10 connected points) than we have for seasonal gradient (3 points in a season). Predatory species were clustered using Ward's hierarchical clustering (Team, 2012). The optimal number of successional clusters was identified by K-means clustering.

Finally, we analyzed whether any competition or IGPIC occurred in succession, and if it could be resolved by species seasonality. We fitted a GLM with season as an environmental variable for each predatory species. We then compared the seasonal trends for species in individual successional clusters. GLMs were fitted as quadratic, to identify temporal optima, and with quasipoisson distribution of error, to eliminate overdispersion. Such GLMs were fitted as species response curves in CANOCO 5 for Windows (Ter Braak & Smilauer, 2012). We then compared the species seasonal trends to identify whether competition or IGPIC could be: a) resolved; b) or not resolved. Contrary to guilds, we considered the negative relationship as resolved if at least one species of the pair had a significant seasonal trend (and therefore avoided at least at some point of the season a species with a non-significant seasonal trend = occurring generally throughout the season).

Results

Sampling results

In total, 4935 predatory individuals, together with 17516 individuals potential prey species were sampled. Singletons were *a priori* excluded from the dataset. Predators consisted primarily of carnivorous Staphylinadae (4500 individuals) with much rarer predatory larvae (333 individuals), parasitoids (various families of Hymenoptera: 72 ind.) and Histeridae (30 ind.). Among the prey species, Hydrophilidae beetles (8419 ind.) and fly larvae (5263 ind.) formed the bulk of prey individuals followed by saprophagous Staphylinidae (Staphylinidae:Oxytelinae: 2508 ind.) and Scarabaeidae (1326 ind.) (Table S1).

The proportion of predators in the community increased throughout succession from 6.1% of total community abundance on day 2 (predator:prey ratio 1:15.4) to ~45% of total abundance on day 11 and 12 (predator:prey ratio 1:1.2) (Fig 1A). Among seasons, the proportion of predators in the community only slightly increased between spring, forming 19% of total community abundance (predator:prey ratio 1:4), and summer seasons (23-24%, predator:prey ratio 1:3) of total community abundance (Fig 1B).

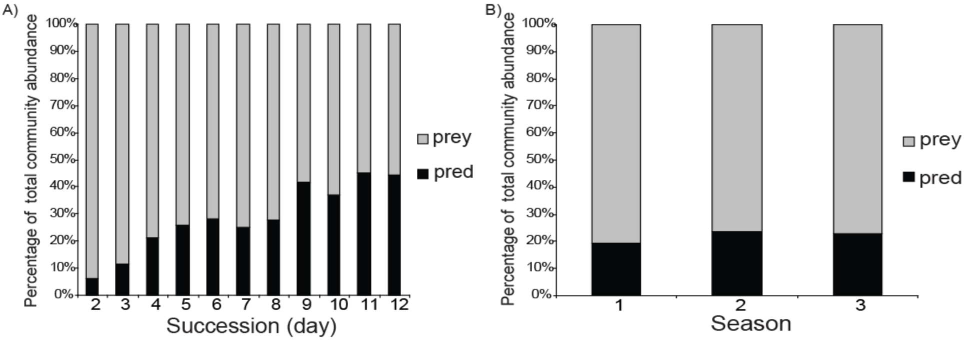

Fig 1: Percentage representation of dung-inhabiting predators (pred) and prey abundance along successional and seasonal gradient.
A) successional gradient, B) seasonal gradient (1 = early spring, 2 = high summer, 3 = late summer/early autumn)

Guilds patterns

Weight clustering of adult beetle species identified 6 predatory guilds and 5 prey guilds (Fig S1). Namely: predatorI) species with dry weight over 100 mg (1 predator, no prey species); predator/preyII) species weighting between 20-35 mg (1 predator, 3 prey species); predator/preyIII) species weighting between 8.5-15 mg (5 predator, 4 prey species); predator/preyIV) species weighting between 3.72-7.9 mg (4 predator, 8 prey species); predator/preyV) species weighting between 1.35-2.9 mg (10 predator, 4 prey species); and predator/preyVI) species weighting with dry weight under 1.15 mg (10 predator, 14 prey species). Finally, by including our assumed larval (2 prey and 1 predatory) and parasitoids' guilds, our analysis included eight predatory guilds (I-VI + larvae + parasitoids) and seven prey guilds (II-VI + large larvae + small larvae). Guild membership of individual species is provided in Table S1. Our eight retrieved predatory guilds represent 28 potential pair interactions all of which show IGPIC (Table 1).

For competition, we found nine trophic links between seven predatory and four prey guilds (Fig 2A). It was not possible to fit the GLMM for predatorI due to its low abundance, and 3 prey guilds (preyII+IV+V) had no significant effect on predatory guilds, or otherwise belonged into their prey spectra. Results of GLMMs are provided in Appendix S1. Out of this guild food web, seven pairs of predatory guilds shared a common prey guild, providing us with seven potential competitive relations for testing (Table 1).

The majority of predatory guilds displayed a significant trend along the successional and seasonal gradient (Fig 2B,C, Appendix S2). All guilds but predIV (although almost marginally significant, $p = 0.08$) displayed a significant successional trend. At the seasonal scale, two guilds did not display a significant trend (predI and predIII with $p = 0.13$ and $p = 0.19$ respectively), two guilds were marginally significant (predV and parasites with $p = 0.05$ and $p = 0.07$ respectively) and the rest of the four guilds eddisplay a significant seasonal trend (Fig 2D,E, Appendix S2).

At the successional scale, 21 out of 28 IGPIC predatory guilds relations could be avoided by successional separation and seven interactions were unresolved (Table 1). If we consider separation or co-occurrence in guild pairs with one or both partners having a marginally significant, yet very different or very similar successional trend, 27 IGPIC relations would be avoided and one could not (co-occurring predIV and predatory larvae). For seven competitive relations, three could be avoided and four were unresolved. Including pairs with marginally significant trends, six competitive relations could be avoided and one could not (again predIV and predatory larvae).

At the seasonal scale, five out of 28 IGPIC relations could be avoided, one could not (predII and predatory larvae, despite significantly avoiding each other in succession) and 22 relations were unresolved (Table 1). Including pairs with marginally significant trends, 11 IGPIC relations could be avoided, four could not (all are significantly avoided in succession) and 13 were unresolved. Out of seven competitive relations, tow could be avoided (including the successional co-occurrence of predIV and predatory larvae) and five were unresolved. Including pairs with marginally significant trends, four relations could be avoided, two could not (again these were avoided significantly in succession) and one was unresolved.

Table 1: Temporal resolution of dung-inhabiting predator guilds: IGPIC and competitive interactions.

avoid = predatory guilds avoid each other along the respective temporal gradient, co-occur = predatory guilds co-occur along the respective temporal gradient, not resolved = one or both guilds in respective pair do not display a significant temporal trend, avoid/co-occur marked with asterisk* = one or both guilds do display a marginally significant trend (p = 0.05-0.1) along the respective temporal gradient, IGPIC = IntraGuild Predation and Interference Competition

predI = species weighting over 100 mg, predatorII = species weighting between 20-35 mg, predatorIII = species weighting between 8.5-15 mg, predatorIV = species weighting between 3.72-7.9 mg, predatorV = species weighting between 1.35-2.9 mg, predatorVI = species weighting with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, Parasites = Hymenoptera parasitoids

Guild pairs		IGPIC	Competition	succession	seasonality
predI	predII	YES	no	avoid	not resolved
	predIII	YES	no	avoid	not resolved
	predIV	YES	no	*avoid	not resolved
	predV	YES	no	avoid	not resolved
	predVI	YES	no	avoid	not resolved
	PredLarv	YES	no	avoid	not resolved
	Parasites	YES	no	avoid	not resolved
predII	predIII	YES	YES	avoid	not resolved
	predIV	YES	no	*avoid	avoid
	predV	YES	no	avoid	*avoid
	predVI	YES	no	avoid	avoid
	PredLarv	YES	no	avoid	co-occur
	Parasites	YES	no	avoid	*co-occur
predIII	predIV	YES	no	*avoid	not resolved
	predV	YES	no	avoid	not resolved
	predVI	YES	no	avoid	not resolved
	PredLarv	YES	no	avoid	not resolved
	Parasites	YES	no	avoid	not resolved
predIV	predV	YES	YES	*avoid	*avoid
	predVI	YES	YES	*avoid	avoid
	PredLarv	YES	YES	*co-occur	avoid
	Parasites	YES	YES	*avoid	*avoid
predV	predVI	YES	YES	avoid	*co-occur
	PredLarv	YES	no	avoid	*avoid
	Parasites	YES	no	avoid	*avoid
predVI	PredLarv	YES	no	avoid	avoid
	Parasites	YES	no	avoid	*avoid
PredLarv	Parasites	YES	YES	avoid	*co-occur

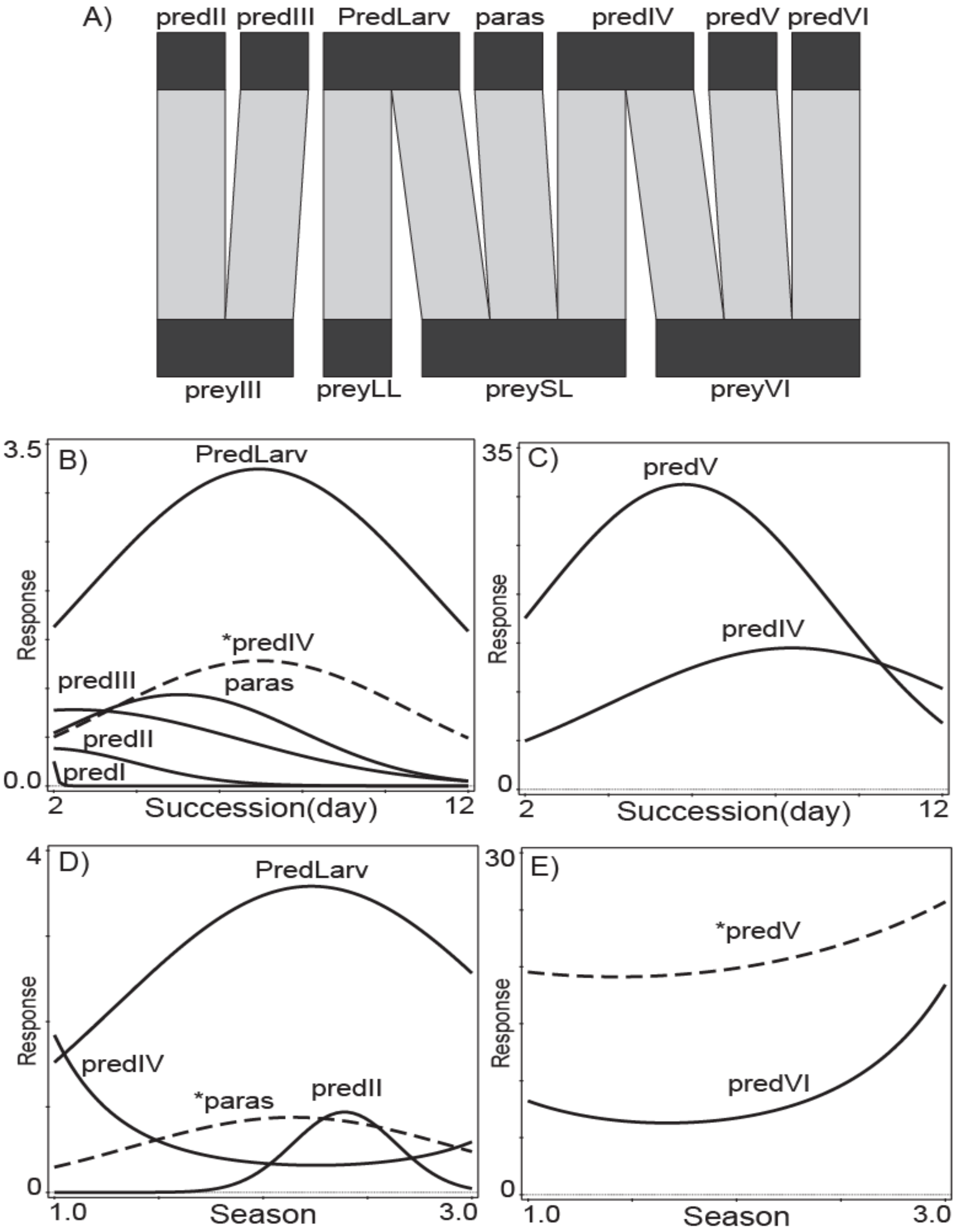

Fig 2: Food web and temporal trends of dung-inhabiting predatory and prey guilds.

A) A food web of dung-inhabiting predatory and prey guilds, B+C) GLMs of predatory guilds along successional gradient, D+E) GLMs of predatory guilds along seasonal gradient. Guilds with marginally significant GLMs (p = 0.05-0.1) in B-E are marked with an asterisk* and are represented by dashed line.

predI = species weighing over 100 mg ,predII = species weighting between 20-35 mg , pred/preyIII = species weighing between 8.5-15 mg, predIV = species weighing between 3.72-7.9 mg, predV = species weighing between 1.35-2.9 mg, pred/preyVI = species weighing with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, paras = Hymenoptera parasitoids, preyLL = prey larvae of Calyptrate Diptera, preySL = prey larvae of Acalyptrate Diptera

Species level patterns

Most predatory species were significantly correlated with at least one prey species from their potential prey spectra (via abundances), although the significant correlations between predators and their prey were generally not very high (mean = 0.32, max = 0.65). The final web consisted of 34 predatory and 33 prey species with a total sum of 186 links. Temporal patterns of predatory and prey species thus resulted in a relatively small number of realized links (connectance = 0.17) and revealed niche partitioning among predatory species (niche overlap = 0.18, mean number of shared partners = 1.53). Among prey, Hydrophilidae beetles received the highest number of links (73), followed by small fly larvae and saprophagous Staphylinidae (37 and 35 respectively), followed by large fly larvae and Scarabaeidae beetles who received the least amount of links (21 and 20 respectively).

The community of dung-inhabiting predators and prey was significantly structured by both the succession (F = 18.7, p = 0.001, first axis explains 12.8% of variability) and season (F = 20.1, p = 0.001, first axis explains 13.6% of variability) (Fig S2). When species are sorted by their temporal optima in the food web, successional and seasonal webs display a differing pattern (Fig 3). The web sorted by successional optima revealed two groups of predators: one early-successional group containing the vast majority of larger predators (I-IV), along with a significant number of predV species; and the second, the late-successional group, containing primarily the smaller predVI species along with less predV and very few large predatory species. The weight of predatory species declines significantly when plotted against species successional optima (simple linear regression; $t = -5.392$, $p < 10^{-6}$, $R^2 = 0.5$). In the web sorted by season, there are also two groups (early - late = spring - summer), however, there is no obvious size separation among adult beetles, reflected in the fact that no trend is apparent when species weights are plotted against their seasonal optima (simple linear regression; $t = 0.524$, $p = 0.604$, $R^2 = 0.009$). Individual species within guilds of adult beetles seems to be evenly distributed, and thus avoid each other along the seasonal gradient. The same applies for parasites species and predatory larvae.

The successional clustering of dung-inhabiting predators resulted in six clusters (A-F; A = very early successional, F = very late successional), of which A contained a single predI species and was therefore omitted from further analyses. The rest of clusters contained six-seven species, and were highly variable with respect to the number of competitive interactions (72 in B, 14 in F), and between 12-19 IGPIC relations (Table 2). The species affiliations to individual clusters are provided in the supplementary material (Fig S3, Appendix S3). The vast majority of species displayed a significant seasonal trend (Appendix S3).

The seasonal separation of species within successional clusters considerably reduced the potential competition and IGPIC. The number of potential competitive relations within successional clusters was significantly lower for all but cluster F (Table 2). In clusters B-E the amount of competitive links in succession decreased by 77-96% when species seasonality was included, while in F cluster only 43% of competitive links could be avoided. For IGPIC, there was a significant seasonal reduction of IGPIC relations in all clusters (Table 2). The amount of IGPIC interactions that could be avoided through seasonality reached between 80-100% (cluster F and E respectively).

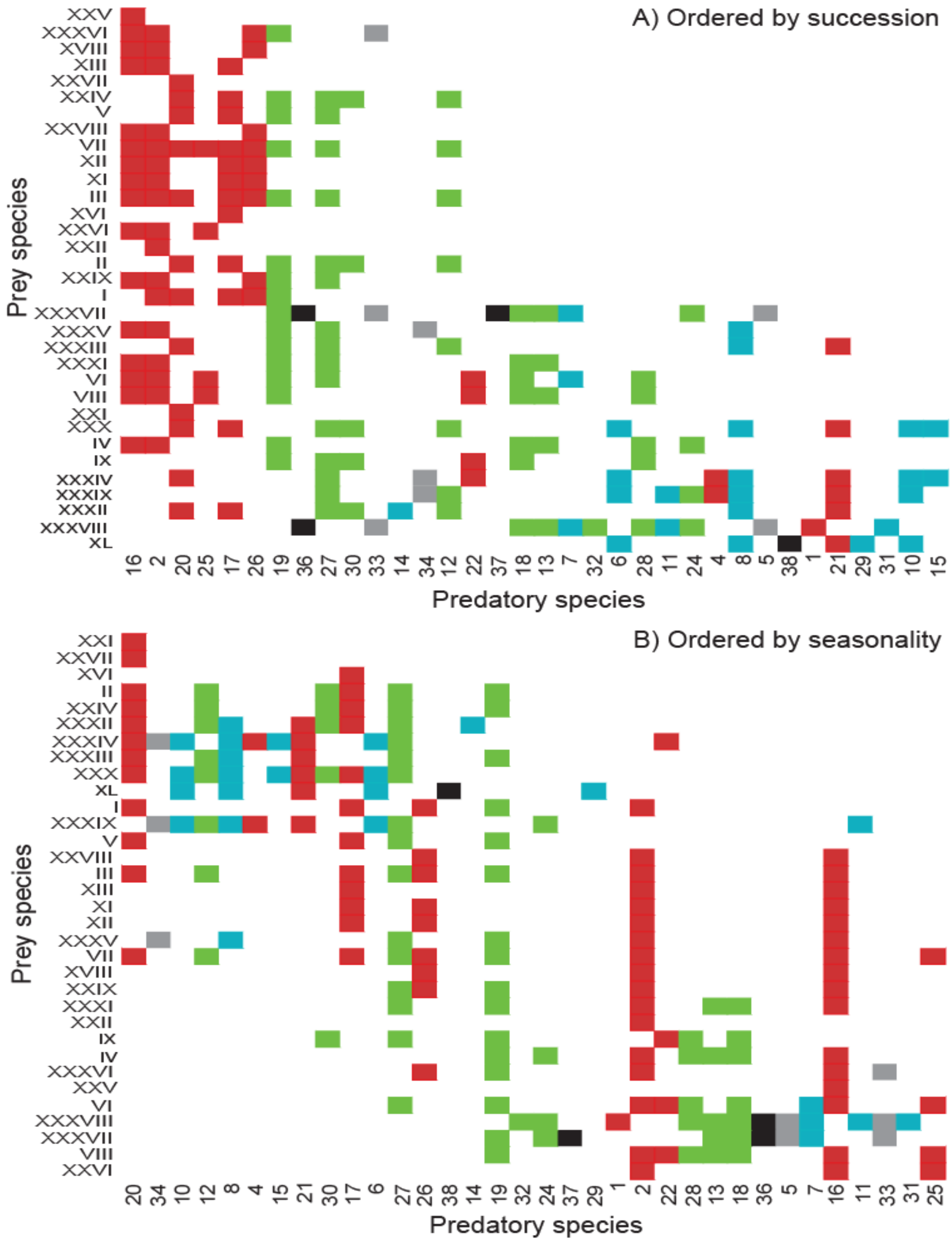

Fig 3: Food web of dung-inhabiting insects

Rows (Roman numerals) represent prey species, columns (Arabic numerals) represent predatory species. Species position in the web (rows

= from up down, columns = from left to right) is established on species successional (A) or seasonal optima (B) (left/up position = very early, right/down position = very late).
red = trophic interaction between prey and "large" predator (size < 3.72 mg; guilds I+II+III+IV), green = with "medium" sized predator (size = 2.9 - 1.135 mg; guild V), blue = "small" predator (size < 1.15 mg; guild VI), grey = predatory larvae and black = parasitoid (guild Parasites)
For individual species abbreviations see Table S1.

Table 2: Number of IGPIC/competitive links in succession and their potential seasonal resolution
Cluster = successional cluster of dung-inhabiting predators (B = early succession, F = very late succession), IGPIC = IntraGuild Predation and Interference Competition. An asterisk* marks significant avoidance of either IGPIC or competitive relations (Chi-square test; unresolved : avoided).

	potential links in succession		unresolved links in season		avoided links in season	
Cluster	competitive	IGPIC	competitive	IGPIC	competitive	IGPIC
B	72	12	16 (22%)	2 (17%)	56 (78%)*	10 (83%)*
C	26	18	1 (4%)	3 (17%)	25 (96%)*	15 (83%)*
D	21	12	3 (14%)	2 (17%)	18 (86%)*	10 (83%)*
E	23	17	4 (17%)	0 (0%)	19 (83%)*	17 (100%)*
F	14	15	8 (57%)	3 (20%)	6 (43%)	12 (80%)*

Discussion

In our study, we have presented new information on the ecology of dung-inhabiting predators, especially with regard to their presumed mutual relations. This provides more insight than simply presenting their successional and seasonal patterns (Sladecek et al., 2013). The proportion of predatory individuals in the community increased throughout succession to almost parity with the prey individuals. In contrast, the predator:prey ratio was virtually constant among seasons. In succession, both predatory guilds and individual species followed a sized-based pattern, large-sized species/guilds were early successional, small sized were late successional. In contrast, there was no sized-based pattern along the seasonal gradient. Temporal segregations in the community of dung-inhabiting predators, i.e. a combination of successional turn-over in species and seasonality, should greatly enhance species coexistence highly reducing or even eliminating IGPIC and competitive relations.

Environmental and niche interpretations of temporal trends in predatory dung insects

The combination of succession in dung predator species and seasonality seems to promote their coexistence via straightforward niche differentiation, as noted in earlier studies (Hanski & Koskela, 1977; Hanski & Koskela, 1979). However, our results suggest that both their succession and seasonality might in fact be driven partly or solely by habitat filtering.

The signature of habitat filtering is most obvious in successional development. The first evidence is the increasing trend in the proportion of predators in the community, which should increase the density of negative interactions among predators due to their aggregation, rather then decrease, which is the main prediction of niche differentiation (Schoener, 1974; Silvertown, 2004). The second line of evidence comes from the separation between "large" and "small" predators along the successional gradient. Although this phenomenon could be interpreted as a mechanism for avoiding IGPIC and interference competition, the aggregation of species with similar traits is the definition of habitat filtering (Keddy, 1992; Kraft et al., 2015). It also includes aggregation of species with similar niches, again in contrast to niche differentiation (MacArthur & Levins, 1967; Schoener, 1974). Size-based habitat filtering is even more strongly supported by guilds level trends, as succession seems to almost perfectly lower the potential for IGPIC and exploitative competition.

The first explanation for such sized based successional patterns could be related to the general lethality of very moist dung for beetles (Whipple et al., 2013). Although there is no specific evidence, the most abundant predators of Staphylinidae are mostly habitat generalists, inhabiting dung, carrion, humus etc. (Freude et al., 1964, 1974; Matuszewski et al., 2010; Sladecek et al., 2013). The only exception are the very early successional species, which could be more tolerant to moist dung, as they occur in early successional dung in almost all studies (e.g. Hanski & Koskela, 1977; Sladecek et al., 2013), as well as in other ephemeral habitats like carrion (Kocarek, 2003; Matuszewski et al., 2011). The majority of predators, however, colonize dung in later development stage (Koskela, 1972) when it more closely resemble humus and has lost a greater portion of its moisture (Gittings & Giller, 1998). Another explanation could be that large early successional predators would be at a disadvantage in the cramped dried out galleries of old dung (Skidmore, 1991), rather than in fresh dung, where their movement is probably less hindered and prey species have fewer hiding places.

In contrast, the seasonal trends should predominantly represent the patterns of niche differentiation. The almost equal ratios of predators and prey among seasons strongly hints at an the interchange of predatory species to lower potential negative interactions. The interchange of species is the most obvious from the seasonal food web, as species of similar sizes are seasonally separated. Finally, seasonality is weak among guilds, while it significantly lowered the potential for IGPIC and competition among individual species. This would again support the idea that niche differentiation should promote coexistence among individual species inside larger groups, here guilds (Arellano et al., 2016; de Camargo et al., 2016; Sladecek et al., 2017b). Nevertheless, we can not fully dismiss the effect of habitat filtering in season, as the species interchanges could be partly driven by their temperature tolerances, as it is in their saprophagous prey species (Landin, 1961).

IGPIC, competition and food webs

To date IGP and interference competition has not been specifically targeted in dung-inhabiting predators, although existing evidence suggests that an increase in predator abundance beyond a certain predator:prey ratio (1:17 - 1:25) does not lead to an increase in predation (Fincher, 1995; Roth, 1982), strongly hinting some negative predators' interactions. Such ratios were achieved on day two of succession and in all seasons in our study. However, temporal displacements should lead to strong reduction of potential IGP and interference competition. The negative interactions unresolved by temporal segregations might be resolved by spatial segregations between temporally co-occurring species (Hanski & Koskela, 1979; Wu et al., 2011). Dung community would therefore follow the same pattern like other communities avoiding negative interactions via space and time, namely carrion-inhabiting communities (Galindo et al., 2016), dragonflies (Crumrine, 2005; Harabis et al., 2012) or mammalian predators (Bischof et al., 2014; Droge et al., 2017).

Exploitative competition is thought to have very little effect on predator coexistence (Valiela, 1974). Our results generally support that idea, as competitive links were mostly significantly omitted by temporal species level trends. The only exception was the cluster of very late successional species. Those, however, might prey heavily upon mites and Collembola, both of which are very abundant during the late parts of dung development and were not included in our study (Laurence, 1954), thus weakening the potential competition between for beetle and fly larvae prey. Nevertheless, there is very little evidence on how much prey dung-inhabiting predators can consume, especially the very large early successional ones. The only study covering such a topic revealed relatively high levels of predation, for a predator which would fall in our predV guild (Valiela, 1969). Such high voracity, amplified by the size of other early successional predators, could completely change the view on limitless amount of prey and therefore the view on importance of exploitative competition.

The creation of a dung-inhabiting community food web was a side goal in our study that should primarily create a hypothesis on the prevalence of competition among dung-inhabiting predators. However, as there are very few food webs published for the dung-inhabiting community (Valiela, 1974; Walsh & Cordo, 1997), we feel obliged to at least comment on several patterns occurring in our hypothetical, yet highly probable, web. Our approach to the food web's creation reflected the general need of time-specific webs (Rasmussen et al., 2013; Valverde et al., 2016), which unlike static general webs present only the trophic relations that can really occur in real world due to predator and prey co-occurrence (Olesen et al., 2008). Although the result is technically a static web instead of multiple webs for each successional day or season, our approach using predator and prey correlations could be used as an example how to create a static web with time-specificity already implemented. In contrast to former studies, the larvae of flies (large and small larvae in our study), seem not to be the most frequent prey (Valiela, 1974), as Hydrophilidae beetles received the most links. In greater contrast to studies focusing on their bio control (e.g. Roth et al., 1988), the larvae of Calyptrate flies (large larvae) seem not to be very frequent prey for dung-inhabiting predators, probably due to the fact that they finish their development before the majority of predators arrive. However, our study did not contain eggs of such flies, which could be preyed on more than larvae (Fincher, 1995; Roth, 1982). Finally, our results should pinpoint the importance of saprophage beetles (Staphylinidae, Scarabaeidae), as potential prey for dung-inhabiting predators, since predation this guild is usually overlooked (Young, 2015).

Study limitations and future perspectives

To the best of our best knowledge our study is the first study to explore the potential role of temporal trends in the elimination of negative relations among dung-inhabiting predators, and as such their coexistence. We are also very well aware of the limitations of our study. The biggest limitation is the theoretical establishment of trophic links, although size based trophic links are generally used in other systems (Woodward et al., 2005). The main purpose of this study is therefore to establish a hypothetical background for future direct studies of trophic interactions among dung-inhabiting organisms, as well as in other ephemeral habitats like carrion.

The future directions of studies focusing on dung-inhabiting insects should therefore focus on:

1) Establishing "real" food web relations between predators and prey, preferably based on feeding trials or metabarcoding, including how much of prey an individual predatory species can process.

2) Using the same feeding trials to establish IGP and interference relations between dung-inhabiting predators.

3) Analyzing the effects of predators on prey species, whether temporal prey trends could be a result of predator avoidance.

4) Exploring the environmental filtering effect of dung moisture and chemistry on individual predatory species.

5) Studying other ephemeral habitats with similarly strong temporal patterns, like carrion, to establish if our findings have a more general ecological perspective.

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. 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). STS acknowledges funding from and a

Univ. of South Bohemia Postdoc project (reg.no. CZ.1.07/2.3.00/30.0006) and the Grant Agency of the Czech Republic (grant number 15-24571S).

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Supporting information

Table S1: Species sampled, their abundances, weights and guilds affiliations

Number = species abbreviations used throughout the study, Sum = species overall abundance, Weight(mg) = average dry weight of 20 individuals of particular species in miligrams, Guild = species guild affiliation (predI = species weighting over 100 mg, pred/preyII = species weighting between 20-35 mg, pred/preyIII = species weighting between 8.5-15 mg, pred/preyIV = species weighting between 3.72-7.9 mg, pred/preyV = species weighting between 1.35-2.9 mg, pred/preyVI = species weighting with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, Parasites = Hymenoptera parasitoids)

	Number	Sum	Weight(mg)	Guild
PREDATORS				
Coleoptera				
Histeridae				
Atholus duodecimstriatus	1	6	8,545	predIII
Hister unicolor	2	14	26,920	predII
Margarinotus purpurascens	3	4	5,538	predIV
Margarinotus ventralis	4	6	8,545	predIII
Hydrophilidae				
Sphaeridinae				
Sphaeridium spp. larvae	5	211		PredLarv
Staphylinidae				
Aleocharinae				
Autalia rivularis	6	222	0,126	predVI
Atheta longicornis	7	559	0,287	predVI
Atheta sp 1	8	90	0,225	predVI
Atheta sp 2	9	475	0,127	predVI
Atheta sp 3	10	24	0,162	predVI
Atheta sp 4	11	49	0,085	predVI
Aleochara brevipennis	12	22	1,580	predV
Aleochara intricata	13	248	1,685	predV
Aleochara sparsa	14	14	1,235	predVI
Paederinae				
Rugilus similis	15	10	0,544	predVI
Staphylininae				
Emus hirtus	16	3	107,234	predI
Ontholestes murinus	17	13	12,290	predIII

	Number	Sum	Weight(mg)	Guild
Philonthus coprophilus	18	975	1,360	predV
Philonthus cruentatus	19	307	2,205	predV
Philonthus marginatus	20	23	3,725	predIV
Philonthus politus	21	54	5,725	predIV
Philonthus rectangulus	22	41	5,114	predIV
Philonthus rotundicollis	23	12	2,009	predV
Philonthus sanguinolentus	24	170	2,156	predV
Philonthus spinipes	25	3	14,780	predIII
Philonthus splendens	26	28	10,590	predIII
Philonthus varians	27	517	1,598	predV
Philonthus varius	28	513	1,587	predV
Tachyporinae				
Cilea silphoides	29	10	0,225	predVI
Tachinus signatus	30	12	1,804	predV
Xantholininae				
Gyrohypnus angustatus	31	16	0,580	predVI
Gyrohypnus fracticornis	32	90	1,595	predV
Diptera				
Muscidae				
Muscinae				
Mesembrina meridiana	33	78		PredLarv
Mydaeinae spp.	34	44		PredLarv
Hymenoptera				
Diapriidae spp.	35	4		Parasites
Figitidae spp.	36	60		Parasites
Pteromalidae spp.	37	2		Parasites
Scelionidae spp.	38	6		Parasites

	Number	Sum	Weight(mg)	Guild
PREY				
Coleoptera				
Hydrophilidae				
Sphaeridinae				
Cercyon castaneipennis	I	151	1,896	preyV
Cercyon haemorrhoidalis	II	153	0,903	preyVI
Cercyon impressus	III	157	1,437	preyV
Cercyon lateralis	IV	1360	0,776	preyVI
Cercyon melanocephalus	V	238	0,592	preyVI
Cercyon pygmaeus	VI	1055	0,161	preyVI
Cercyon quisquilius	VII	384	0,505	preyVI
Cryptopleurum crenatum	VIII	1008	0,530	preyVI
Cryptopleurum minutum	IX	3138	0,390	preyVI
Megasternum concinuum	X	3	0,356	preyVI

	Number	Sum	Weight(mg)	Guild
Sphaeridium bipustulatum	XI	278	3,866	preyIV
Sphaeridium lunatum	XII	320	10,110	preyIII
Sphaeridium scarabaeoides	XIII	174	10,785	preyIII
Scarabaeidae				
Scarabaeinae				
Onthophagus coenobita	XIV	6	21,930	preyII
Onthophagus joanne	XV	87	5,574	preyIV
Onthophagus ovatus	XVI	292	6,793	preyIV
Aphodiinae				
Aphodius ater	XVII	2	4,980	preyIV
Aphodius erraticus	XVIII	16	7,912	preyIV
Aphodius fimetarius	XIX	428	9,815	preyIII
Aphodius fossor	XX	38	28,950	preyII
Aphodius granarius	XXI	3	2,945	preyV
Aphodius haemorrhoidalis	XXII	247	2,596	preyV
Aphodius prodromus	XXIII	26	4,898	preyIV
Aphodius pusillus	XXIV	16	1,150	preyVI
Aphodius rufipes	XXV	3	34,600	preyII
Aphodius rufus	XXVI	116	7,480	preyIV
Aphodius sphacelatus	XXVII	42	3,657	preyIV
Aphodius subterraneus	XXVIII	4	10,455	preyIII
Staphylinidae				
Oxytelinae				
Anotylus rugosus	XXIX	125	0,644	preyVI
Anotylus tetracarinatus	XXX	1507	0,093	preyVI
Platystethus arenarius	XXXI	769	0,395	preyVI
Proteninae				
Megarthrus denticollis	XXXII	30	0,188	preyVI
Megarthrus depressus	XXXIII	77	0,210	preyVI
Diptera				
Anthomyiidae spp.	XXXIV	175		
Muscidae				
Musca autumnalis	XXXV	3949		LL
Sarcophagidae				
Ravinia pernix	XXXVI	250		LL
Sepsidae				
Saltella nigripes	XXXVII	78		SL
Saltella spondylii	XXXVIII	702		SL
Sepsis spp.	XXXIX	86		SL
Sphaeroceridae spp.	XL	23		SL

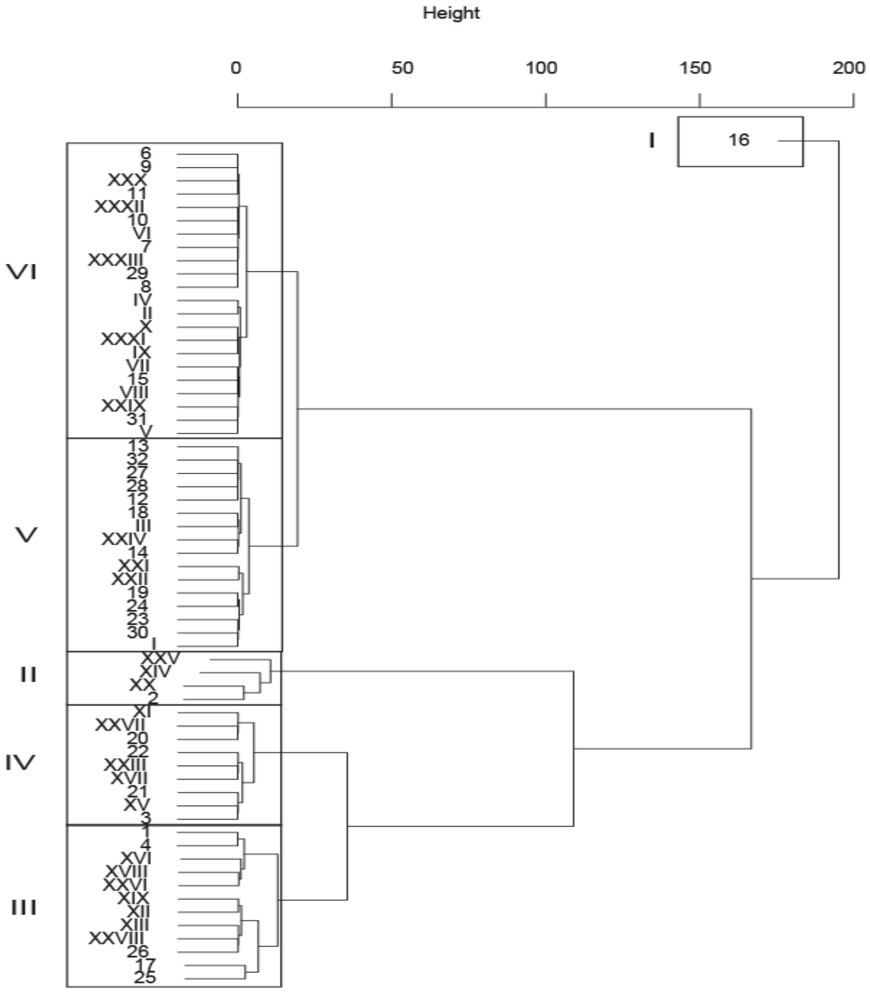


Fig S1: Sized-based clustering of adult dung-inhabiting beetles

The clustering was done using Ward's hiearchical clustering in R 2.15.2. Number or clusters and clusters themselves were identified by K-means clustering. Clusters retrieved here represent the predatory guilds throughout the study.

I = species weighting over 100 mg, II = species weighting between 20-35 mg, III = species weighting between 8.5-15 mg, IV = species weighting between 3.72-7.9 mg, V = species weighting between 1.35-2.9 mg, VI = species weighting with dry weight under 1.15 mg

Roman numerals denote prey species, Arab numerals denote predatory species

For individual species abbreviations see Table S1

Appendix S1: Results of GLMMs analyzing the dung-inhabiting predators guilds affinity to prey guilds.

The prey guilds that significantly positively affected predator guild and belong to its prey spectrum (predator hunts same sized or smaller prey) are marked by grey color. Such guilds were linked to respective predator guild in guild food web.

pred/preyII = species weighting between 20-35 mg , pred/preyIII = species weighting between 8.5-15 mg, pred/preyIV = species weighting between 3.72-7.9 mg, pred/preyV = species weighting between 1.35-2.9 mg, pred/preyVI = species weighting with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, Parasites = Hymenoptera parasitoids, preyLL = prey larvae of Calyptrate Diptera, preySL = prey larvae of Acalyptrate Diptera

predII + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']

Family: poisson ( log )
Formula: predII ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL + (1 |
day) + (1 | block) + (1 | SP)
   Data: predprey

     AIC      BIC   logLik deviance
 81.6301 113.3409 -29.8151  59.6301

Random effects:
 Groups  Name        Variance  Std.Dev.
 day     (Intercept) 9.194e-06 0.003032
 block   (Intercept) 1.287e+00 1.134259
 SP      (Intercept) 1.646e-05 0.004058
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
              Estimate   Std. Error  z value Pr(>|z|)
(Intercept) -4.732369   1.021338  -4.633  3.6e-06
preyII      -0.086399   0.373565  -0.231  0.81709
preyIII      0.094967   0.035719   2.659  0.00784
preyIV      -0.150841   0.071836  -2.100  0.03575
preyV        0.091449   0.047572   1.922  0.05457
preyVI       0.004496   0.004605   0.976  0.32887
```

```
preyLL       0.005493   0.003035   1.810  0.07028
preySL       0.026841   0.015658   1.714  0.08649
```

predIII + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']

Family: poisson ( log )
Formula: predIII ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL + (1
| day) + (1 | block) + (1 | SP)
   Data: predprey

      AIC       BIC    logLik  deviance
 237.3915  269.1023 -107.6958  215.3915

Random effects:
 Groups Name        Variance  Std.Dev.
 day    (Intercept) 2.022e-07 0.0004497
 block  (Intercept) 1.104e-01 0.3322718
 SP     (Intercept) 6.618e-02 0.2572488
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.710404   0.351016  -4.873  1.1e-06
preyII      -0.208851   0.244332  -0.855   0.3927
preyIII      0.028760   0.014717   1.954   0.0507
preyIV      -0.003641   0.014636  -0.249   0.8035
preyV        0.025656   0.023127   1.109   0.2673
preyVI       0.002770   0.001543   1.795   0.0727
preyLL       0.001882   0.001377   1.367   0.1716
preySL       0.013602   0.009381   1.450   0.1471
```

predIV + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
Family: poisson ( log )
Formula: predIV ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL + (1
| day) + (1 | block) + (1 | SP)
   Data: predprey

      AIC       BIC    logLik  deviance
 325.5986  357.3095 -151.7993  303.5986

Random effects:
 Groups Name        Variance  Std.Dev.
 day    (Intercept) 2.270e-01 4.764e-01
 block  (Intercept) 7.031e-11 8.385e-06
 SP     (Intercept) 7.197e-01 8.484e-01
Number of obs: 132, groups: day, 11; block, 4; SP, 3
```

```
Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.627364   0.554224  -1.132  0.25765
preyII       0.150264   0.159201   0.944  0.34524
preyIII      0.013812   0.021110   0.654  0.51291
preyIV       0.002370   0.009474   0.250  0.80245
preyV       -0.093103   0.031209  -2.983  0.00285
preyVI       0.003309   0.001013   3.267  0.00109
preyLL      -0.004844   0.003089  -1.568  0.11691
preySL       0.017437   0.007649   2.279  0.02264
```

predV + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']

Family: poisson ( log )
Formula: predV ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL + (1 |
day) + (1 | block) + (1 | SP)
   Data: predprey

      AIC       BIC    logLik  deviance
1245.9109 1277.6218 -611.9555 1223.9109

Random effects:
 Groups Name        Variance  Std.Dev.
 day    (Intercept) 0.1797242 0.42394
 block  (Intercept) 0.0009977 0.03159
 SP     (Intercept) 0.0326771 0.18077
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.8301979  0.1716309  16.490  < 2e-16
preyII       0.1325778  0.0283659   4.674 2.96e-06
preyIII      0.0101611  0.0036282   2.801  0.00510
preyIV      -0.0036081  0.0024849  -1.452  0.14651
preyV       -0.0040995  0.0048782  -0.840  0.40071
preyVI       0.0007273  0.0002225   3.269  0.00108
preyLL      -0.0001018  0.0003513  -0.290  0.77211
preySL       0.0022214  0.0014705   1.511  0.13088
```

predVI + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']

Family: poisson ( log )
Formula: predVI ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL + (1
| day) + (1 | block) + (1 | SP)
```

```
   Data: predprey

     AIC      BIC   logLik deviance
1175.066 1206.777 -576.533 1153.066

Random effects:
 Groups Name        Variance Std.Dev.
 day    (Intercept) 0.125518 0.35429
 block  (Intercept) 0.009059 0.09518
 SP     (Intercept) 0.262221 0.51207
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  2.0440657  0.3251031   6.287 3.23e-10
preyII       0.1583174  0.0483113   3.277  0.00105
preyIII      0.0371880  0.0064495   5.766 8.12e-09
preyIV      -0.0239168  0.0047932  -4.990 6.04e-07
preyV       -0.0180119  0.0098415  -1.830  0.06722 .
preyVI       0.0015295  0.0002654   5.762 8.29e-09
preyLL      -0.0013173  0.0006269  -2.101  0.03562
preySL       0.0006851  0.0022997   0.298  0.76577
```

PredLarv + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']

Family: poisson ( log )
Formula: PredLarv ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL +
(1 | day) + (1 | block) + (1 | SP)
   Data: predprey

      AIC       BIC    logLik  deviance
 561.8468  593.5577 -269.9234  539.8468

Random effects:
 Groups Name        Variance Std.Dev.
 day    (Intercept) 0.06937  0.2634
 block  (Intercept) 0.07848  0.2801
 SP     (Intercept) 0.08827  0.2971
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)  0.7082297  0.2605924   2.718  0.00657
preyII       0.1391931  0.0740669   1.879  0.06021
preyIII     -0.0003585  0.0109305  -0.033  0.97383
preyIV      -0.0061092  0.0082164  -0.744  0.45716
preyV       -0.0564201  0.0173202  -3.257  0.00112
preyVI       0.0014493  0.0006935   2.090  0.03665
```

```
preyLL       0.0025263  0.0009472   2.667  0.00765
preySL       0.0104262  0.0035724   2.919  0.00352
```

Parasites + prey guilds:

```
Generalized linear mixed model fit by maximum likelihood ['glmerMod']
 Family: poisson ( log )
Formula: Parasites ~ preyII + preyIII + preyIV + preyV + preyVI + preyLL + preySL +
(1 | day) + (1 | block) + (1 | SP)
   Data: predprey

      AIC       BIC    logLik  deviance
 282.1064  313.8172 -130.0532  260.1064

Random effects:
 Groups Name        Variance  Std.Dev.
 day    (Intercept) 1.974e-02 1.405e-01
 block  (Intercept) 4.925e-09 7.018e-05
 SP     (Intercept) 7.443e-02 2.728e-01
Number of obs: 132, groups: day, 11; block, 4; SP, 3

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept) -1.0707774  0.2765264  -3.872 0.000108
preyII      -0.1078643  0.1733385  -0.622 0.533761
preyIII     -0.0348145  0.0174656  -1.993 0.046227
preyIV      -0.0005689  0.0144982  -0.039 0.968700
preyV        0.0810767  0.0212866   3.809 0.000140
preyVI       0.0019842  0.0012559   1.580 0.114115
preyLL      -0.0037254  0.0020246  -1.840 0.065759
preySL       0.0195450  0.0057018   3.428 0.000608
```

Appendix S2: Results of GLMs analyzing dung-inhabiting predators successional and seasonal trends.

Marginally significant results (p = 0.05-0.1) are marked with grey color, insignificant results (p > 0.1) are marked by an asterisk*.

predatorII = species weighting between 20-35 mg , predatorIII = species weighting between 8.5-15 mg, predatorIV = species weighting between 3.72-7.9 mg, predatorV = species weighting between 1.35-2.9 mg, predatorVI = species weighting with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, Parasites = Hymenoptera parasitoids

Succession:

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p	Optimum
predI	quadratic	63.4	64.7	<0.00001	-
predII	quadratic	34.0	15.8	<0.00001	1.82
predIII	quadratic	15.4	8.7	0.0003	2.49
predIV	quadratic	4.7	2.5	0.08263	6.97
predV	quadratic	38.4	40.3	<0.00001	5.81
predVI	quadratic	10.2	6.3	0.00251	8.40
PredLarv	quadratic	5.4	3.7	0.02657	6.95
Parasites	quadratic	13.4	7.9	0.00058	5.02

Season:

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p	Optimum
*predI	quadratic	12.2	2.1	0.12615	2.45
predII	quadratic	27.8	13.0	<0.00001	2.39
*predIII	quadratic	3.4	1.7	0.1939	0.904
predIV	quadratic	23.4	16.8	<0.00001	-
predV	quadratic	4.3	3.0	0.05113	-
predVI	quadratic	25.7	22.5	<0.00001	-
PredLarv	quadratic	10.2	6.9	0.0014	2.23
Parasites	quadratic	6.5	2.8	0.06723	2.14

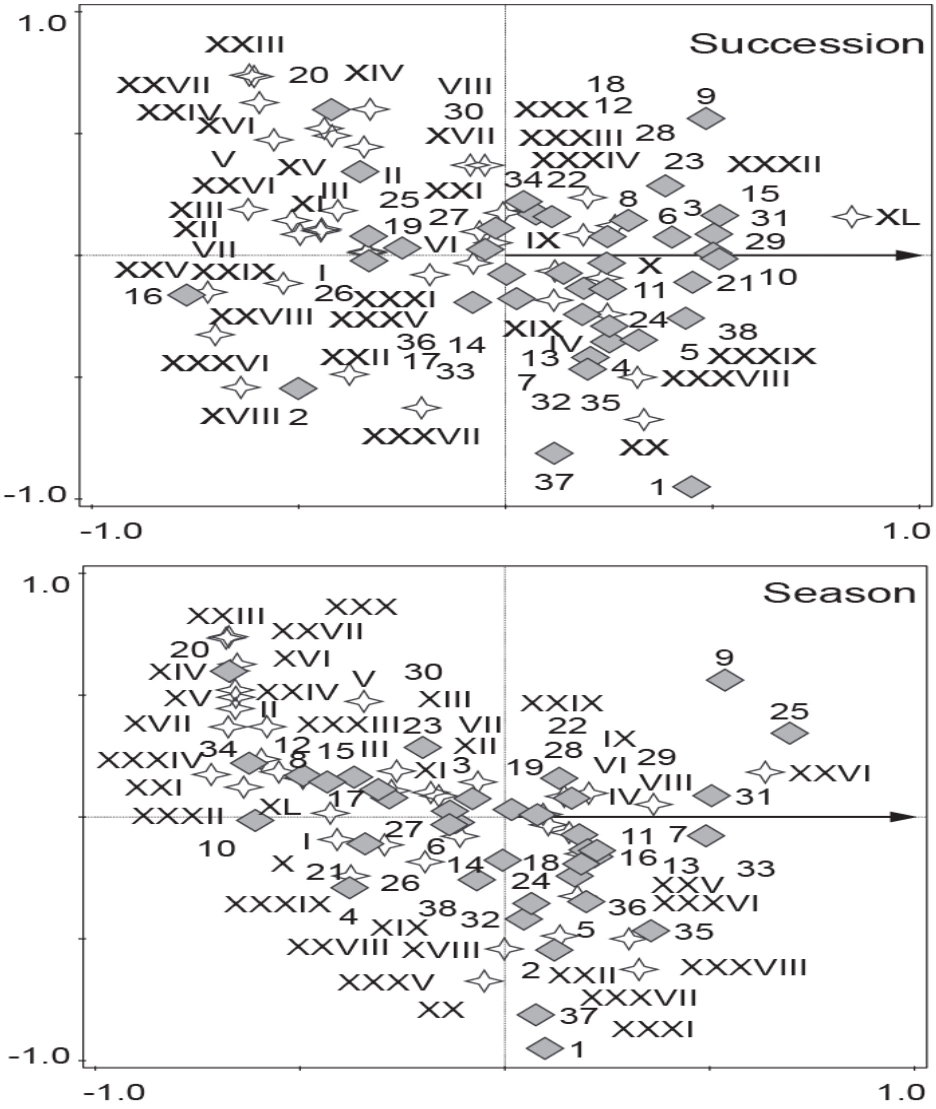


Fig S2: Ordination diagrams of dung-inhabiting insects' succession and seasonality

Both diagrams were created in CANOCO 5 for Windows as results of significant analyses of dung-inhabiting species succession (Succession; F = 18.7, p = 0.001, first axis explains 12.8% of variability) and seasonality (Season; F = 20.1, p = 0.001, first axis explains 13.6% of variability). Stars = prey species, Diamonds = predatory species For species abbreviations see Table S1.

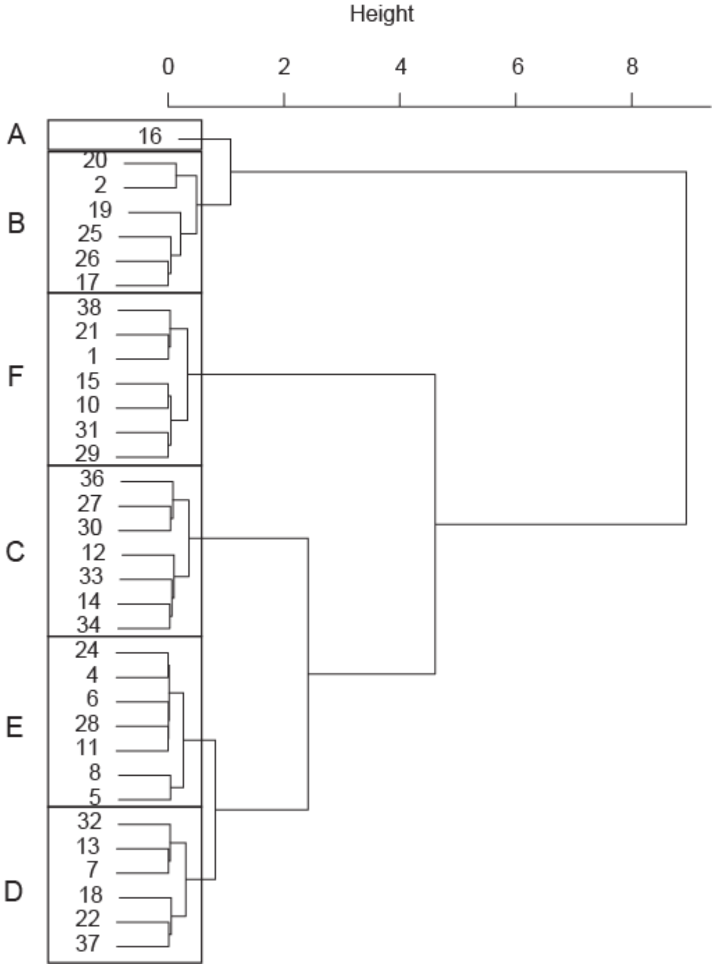


Fig S3: Clustering of dung-inhabiting predators along successional gradient.

The clustering is based on species optima from CCA analyzing their successional turn-over. Clustering was done using Ward's hierarchical clustering in R 2.15.2. Number of clusters and clusters themselves were identified by K-means clustering. For individual species abbreviations see Table S1.

Appendix S3: Species seasonality vs. IGP and competition in successional clusters

Cluster = species affiliation to a successional cluster (A = very early successional, F = very late successional), Number = species code (established in Table S1), Guild = species affiliation to a size based guild (predI = species weighting over 100 mg ,predII = species weighting between 20-35 mg , predIII = species weighting between 8.5-15 mg, predIV = species weighting between 3.72-7.9 mg, predV = species weighting between 1.35-2.9 mg, predVI = species weighting with dry weight under 1.15 mg, PredLarv = predatory larvae of beetles and flies, Parasites = Hymenoptera parasitoids)

IGP = table of potential IGP relations among predators in particular successional cluster (1 = potential IGP relation; rows = species that can IGP on the particular predator, columns = species that the particular predator can IGP on), Competition = table of potential competitive relations among predators in particular successional cluster (number in cells denote number of competitive links in each pair), greyed out numbers = IGP/competition could be avoided via species seasonality, numbers with white background = IGP/competition could not be avoided via species seasonality

GLM results = results of GLMs fitted for species seasonal trends, GLM trends = fitted GLMs (only for species with significant seasonality)

Cluster	Number	Species	Guild
A	16	*Emus hirtus*	predI
B	2	*Hister unicolor*	predII
B	20	*Philonthus marginatus*	predIV
B	19	*Philonthus cruentatus*	predV
B	25	*Philonthus spinipes*	predIII
B	17	*Ontholestes murinus*	predIII
B	26	*Philonthus splendens*	predIII
C	36	Figitidae spp.	Parasites
C	27	*Philonthus varians*	predV
C	30	*Tachinus signatus*	predV
C	33	*Mesembrina meridiana*	PredLarv
C	14	*Aleochara sparsa*	predVI

C	34	Mydaeinae spp.	PredLarv
C	12	*Aleochara brevipennis*	predV
D	22	*Philonthus rectangulus*	predIV
D	37	Pteromalidae spp.	Parasites
D	18	*Philonthus coprophilus*	predV
D	13	*Aleochara intricata*	predV
D	7	*Atheta longicornis*	predVI
D	32	*Gyrohypnus fracticornis*	predV
E	6	*Autalia rivularis*	predVI
E	28	*Philonthus varius*	predV
E	11	*Atheta sp 4*	predVI
E	24	*Philonthus sanguinolentus*	predV
E	4	*Margarinotus ventralis*	predIII
E	8	*Atheta sp 1*	predVI
E	5	*Sphaeridium* spp. larvae	PredLarv
F	38	Scelionidae spp.	Parasites
F	1	*Atholus duodecimstriatus*	predIII
F	21	*Philonthus politus*	predIV
F	29	*Cilea silphoides*	predVI
F	31	*Gyrohypnus angustatus*	predVI
F	10	*Atheta sp 3*	predVI
F	15	*Rugilus similis*	predVI

Cluster B

IGP

	2	20	25	17	26	19
2						
20	1		1	1	1	
25	1					
17	1					
26	1					
19	1	1	1	1	1	

Competition

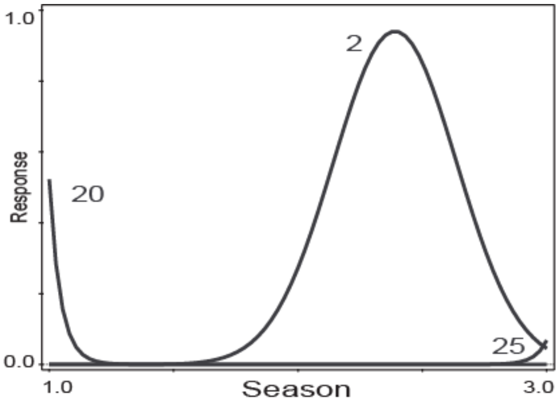
	20	25	17	26	19
2	3	4	6	9	10
20		1	8	3	7
25			1	1	3
17				5	6
26					5

GLM results

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p
19	quadratic	2.0	1.2	0.31687
20	quadratic	43.9	36.3	<0.00001
25	quadratic	29.0	10.4	0.00007
26	quadratic	2.1	0.6289	0.53479
17	quadratic	7.2	2.2	0.11091
2	quadratic	27.8	13.0	<0.00001

GLM trends



Cluster C

IGP

	36	27	30	33	14	34	12
36		1	1		1		1
27							
30							
33	1	1	1		1	1	1
14		1	1				1
34	1		1	1	1		1
12							

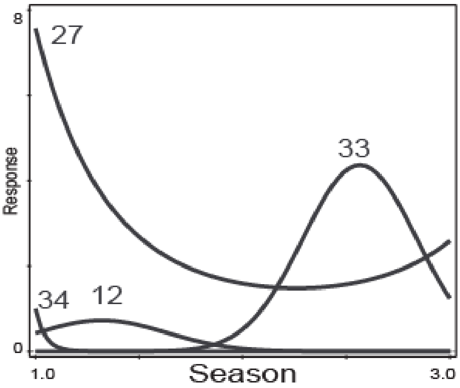
Competition

	27	30	33	14	34	12
36			2			
27		5		1	3	8
30				1		4
33						
14						1
34						1

GLM results

Distribution	quasi-Poisson			
Link function	log			
Response	Type	R2[%]	F	p
27	quadratic	29.1	29.3	<0.00001
30	quadratic	9.2	7.17	0.06909
14	quadratic	2.6	0.8075	0.55178
12	quadratic	7.17	12.5	0.00001
34	quadratic	44.7	37.1	<0.00001
33	quadratic	38.0	38.1	<0.00001
36	quadratic	5.4	1.9	0.15064

GLM trends



Cluster D

IGP

	22	37	18	13	7	32
22						
37	1		1	1	1	1
18	1					
13	1					
7	1		1	1		1
32	1					

Competition

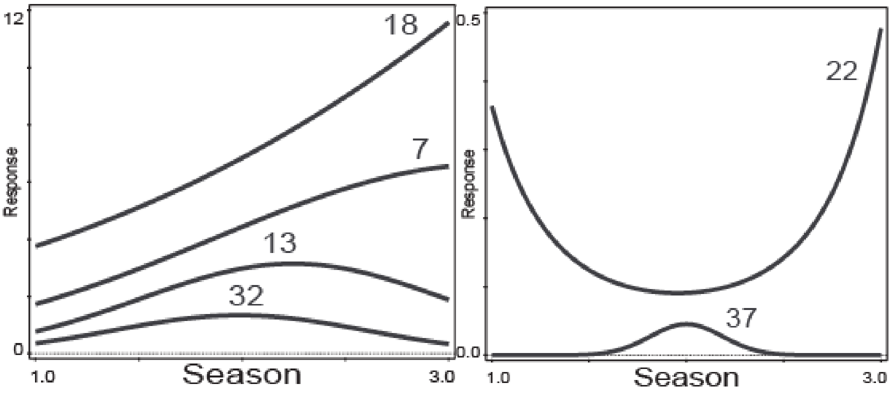
	37	18	13	7	32
22		3	1	1	
37		1	1	1	
18			5	3	1
13				2	1
7					1

GLM results

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p
32	quadratic	21.0	16.0	<0.00001
18	quadratic	20.4	3.17	<0.00001
13	quadratic	15.4	9.0	0.00021
7	quadratic	19.7	15.6	<0.00001
22	quadratic	9.5	4.4	0.01478
37	quadratic	26.2	6.7	0.00163

GLM trends



Cluster E

IGP

	6	28	11	24	4	8	5
6		1		1	1		
28					1		
11		1		1	1		
24					1		
4							
8		1		1	1		
5	1	1	1	1	1	1	

Competition

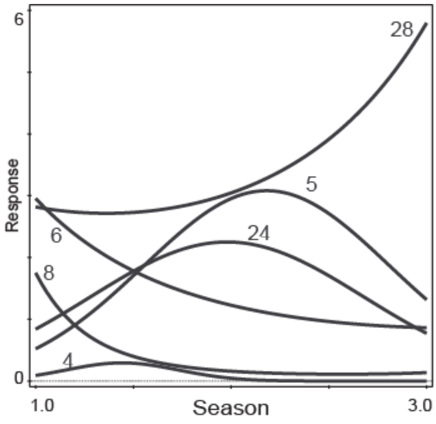
	28	11	24	4	8	5
6		1	1	2	4	
28		1	2			1
11			2	1	1	1
24				1	1	2
4					2	

GLM results

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p
24	quadratic	18.4	14.2	<0.00001
28	quadratic	16.3	13.7	<0.00001
6	quadratic	16.7	10.6	0.00005
8	quadratic	41.3	31.9	<0.00001
11	quadratic	3.4	1.6	0.2107
4	quadratic	14.9	4.4	0.01469
5	quadratic	24.2	17.3	<0.00001

GLM trends



Cluster F

IGP

	38	1	21	29	31	10	15
38		1	1	1	1	1	1
1							
21		1					
29		1	1				
31		1	1				
10		1	1				
15		1	1				

Competition

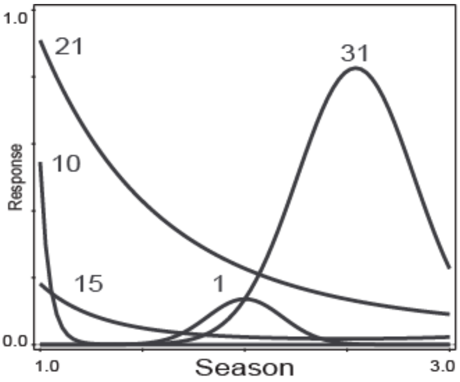
	21	29	31	10	15
38	1	1		1	
1			1		
21		1		4	2
29				1	
31					
10					2

GLM results

Distribution	quasi-Poisson
Link function	log

Response	Type	R2[%]	F	p
31	quadratic	18.2	8.0	0.00054
21	quadratic	21.4	11.5	0.00003
29	quadratic	1.7	0.4618	0.63119
15	quadratic	16.9	4.5	0.01343
10	quadratic	40.8	32.6	<0.00001
1	quadratic	33.1	16.1	<0.00001
38	quadratic	2.6	0.4345	0.64851

GLM trends



CHAPTER IV

Frantisek Xaver Jiri Sladecek, Stefan Dötterl, Irmgard Schäffler, Simon Tristram Segar, Martin Konvicka. *Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds.* (manuscript).

Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds.

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Abstract

Chemical cues play a crucial role in insects' foraging for food and their relations with insects are extensively studied in plant-pollinator systems. In contrast, relations between insect decomposers and their habitats', e.g. dung, chemistry are rather understudied, despite obvious chemical signalization from such habitats. In this study, we therefore focused on succession of volatiles released throughout cow dung pats' ageing and their potential influence on dung-inhabiting insects. Using gas chromatography/mass spectrometry we identified the spectrum and amounts of volatile compounds released from 1 hour, 1, 2, 3, 5 and 7 days old dung. We then compared the volatiles' successional patterns with patterns of dung-inhabiting beetle and fly species. We identified 54 dung emitted volatile compounds. The volatiles formed two large successional groups with turning point at 48 hours old dung. The early successional group consisted primarily of alcohols and phenols, the late one consisted of esters, nitrogen- and sulfur-compounds. Patterns of volatile compounds were almost perfectly reflected by insects' species patterns, as beetles occurred along late successional volatiles, while flies predominantly co-occurred along early successional volatiles. This association of insects' and chemical successional patterns supports the habitat filtering as the assembly rule during insect succession in dung.

Keywords: Diptera, dung beetles,, environmental filtering, ephemeral habitats, temporal segregations

Introduction

Food location is the most crucial ability in animals and is achieved by utilizing either visual, chemical (smell, taste) or both cues (Balkenius et al., 2006; Karmakar et al., 2016; Milet-Pinheiro et al., 2015). Despite our inability for generalization, there is an increasing number of studies reporting that species rely on chemical cues when looking for food, even in taxa previously considered to rely heavily on visual cues (Milet-Pinheiro et al., 2015; Omura & Honda, 2009; Primante & Dotterl, 2010).

Chemical cues are commonly used for locating food sources in both herbivores and saprophages, and also their predators (e.g. de Mendonça et al., 1999; Frederickx et al., 2012b; Hulcr et al., 2006; Segura et al., 2012). The majority of attention is given to the interactions between herbivorous insects and their hosts, especially the interactions between flowering plants and their associated pollinators (e.g. Dotterl et al., 2006). Among pollinators, the chemical clues are utilized primarily by insects (Mitchell et al., 2015; Primante & Dotterl, 2010), with visual clues being more important in birds (Knudsen et al., 2004) or exceptionally in some diurnal moths (Balkenius et al., 2006). Although there is evidently an abundance of studies focusing on the pollinators, there is proportionally less studies focusing on saprophage communities inhabiting habitats even more smelly than flowers, the ephemeral habitats such as dung or carrion. Both dung and carrion are temporally unstable yet energy rich (Finn, 2001).

In contrast to the insects visiting flowering plants, the insects inhabiting ephemeral habitats should be solely attracted by chemical cues (Halffter & Matthews, 1966), particularly because such insects are often attracted to plants that chemically mimic chemicals released by their natural habitats (Jurgens et al., 2006; Jurgens et al., 2013; Marino et al., 2009; Midgley et al., 2015). Up to now, three major topics were studied in ephemeral habitats' chemistry: insect preference for habitat type (e.g., dung types (Dormont et al., 2007)), changes in volatile compounds during habitat ageing (carrion (Perrault et al., 2015)) and insect preference for specific compounds during habitat ageing (carrion (von Hoermann et al., 2011; von Hoermann et al., 2013)). Preference for various dung types (dung produced by different animals) has been a popular topic in dung inhabiting communities, resulting into some fine scale beetle preferences for individual types of herbivore's dung (Dormont et al., 2004; Dormont et al., 2007; Stavert et al., 2014). Changes in volatile compounds along habitat ageing were studied exclusively in carrion (e.g. Perrault et al., 2015); and specifically for purpose of forensic entomology (Paczkowski et al., 2015). However, the picture of succession of carrion emitted volatile compounds along ageing is far from complete, as there are some similarities between those studies (high presence of oligosulfides from early to mid-successional stage onward followed by indole, phenolic, and carboxylic derivates), there are also significant dissimilarities (presence of aldehydes and alcohols in either very early or very late succession) (Dekeirsschieter et al., 2009; Forbes & Perrault, 2014; Paczkowski et al., 2015; Stadler et al., 2015). Only few studies considered also carrion inhabiting insects and determined the responses of these insects towards compounds released during different stages of decomposition. Such studies revealed that early successional burying beetles (Silphidae) (Podskalska et al., 2009) and blowflies (Calliphoridae) (Frederickx et al., 2012b) have a preference for oligosulfides, while late successional hide beetles (Dermestidae) respond to carboxylic derivates (von Hoermann et al., 2011). Blowflies even switch between habitats utilizing volatile compounds as they accept dung emitted volatiles to feed as adults (Erzinclioglu, 1996), but focusing on oligosulfids that mark the relatively fresh carcass when ovipositing (Brodie et al., 2016). There are, however, no studies that would directly match the successional patterns of insects and volatile compounds.

We therefore present here a first study that focuses on both the changes in volatile compounds emitted and the insect successional patterns along habitat ageing, using cow dung and its associated insect community as a model. Dung is inhabited by a wide array of organisms, among which beetles and flies are usually considered to play the main ecological role in dung degradation (Wu & Sun, 2010), either by destroying dung themselves (Slade et al., 2007) or by facilitating the activity of other dung-inhabiting biota, i.e. earthworms (Holter, 1977), fungi (Blackwell & Malloch, 1991; Lussenhop et al., 1980) and bacteria (Stevenson & Dindal, 1987). Contrary to dung volatile compounds, the succession in both beetle (Sladecek et al., 2013) and fly species (Sladecek et al., 2017b) is a well documented fact. As succession of dung-inhabiting insects is probably driven by their environmental affinities (Sladecek et al., 2017a), i.e. habitat filtering (Keddy, 1992; Kraft et al., 2015), the dung emitted volatile compounds could constitute for one of such environmental conditions. The prime evidence could be the stability of species successional sequence throughout the whole temperate region, where species follow the same successional pattern in Europe (e.g. Koskela & Hanski, 1977; Sladecek et al., 2013; Sladecek et al., 2017b), their original home, as well as in Americas, to where the majority of them was introduced (Mohr, 1943; Wassmer, 2014; Wingo et al., 1974). This could indicate that species might have some affinity to chemicals released at some stage of dung development. Secondly, the similar phenomenon, stability of successional patterns and species affinity to specific compounds emitted by ageing habitat, seems to apply in ecologically similar carrion-inhabiting insects (Kalinova et al., 2009; Matuszewski et al., 2011; Tabor et al., 2004; von Hoermann et al., 2011; von Hoermann et al., 2013). Working with beetles, flies and dung volatile compounds, we specifically asked following questions:

1) Is there a succession of dung volatile compounds along the dung pat's ageing?

2) Are there correlations between the abundance and species richness of dung-inhabiting insects and the amount and number of volatiles during ageing of dung pats?
3) Is there trend between the composition of the insect fauna and the volatile patterns released during succession?

Material and Methods

Study site

The study was carried out on a 23 ha pasture, 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).

Volatiles sampling

Sampling of dung volatiles took place in June 2015 (22 – 29th June). Dung volatiles were sampled from artificially created cow dung pats of 1.5 litres volume. Fresh, just defecated dung was sampled from several stalled cows and homogenized. Five dung pats were then created at the study site, forming a line with each pat placed 10 m apart. Each of those pats represented one replicate. Clean metallic tools were used to manipulate the dung in order to avoid / minimize contamination with non-faecal volatiles. Dung volatiles were sampled repeatedly from those five pats at 1, 24, 48, 72, 120 and 168 hours of dung age resulting in 30 samples.

Sampling of dung pat volatiles was conducted by placing a glass funnel of 9 cm diameter airtight on the surface of a dung pat. The dung volatiles were then trapped into an adsorbent tubes (quartz microvials, 15 mm length, 2 mm inner diameter) filled with 1.5 mg of Tenax-TA 60–80 and 1.5 mg of Carbotrap 20–40, fixed in the tube with glass wool). Air with dung volatiles was sucked into those tubes with a 9V operated membrane pump (G12/01 EB, Rietschle Thomas, Puchheim, Germany) (Dotterl et al., 2006; Mitchell et al., 2015). The flow was adjusted to 200 ml.min^{-1} by a flow meter. Blank control samples (two per successional stage) were sampled from the meadow using the same setup, however, the glass funnel did not touch the grass (sampling took place ~ 10 cm above ground). Samples for 1 hour old dung (released high amounts of volatiles) were sampled for 5 minutes only, while all other samples an blanks were sampled for 10 minutes.

Chemical analyses

Volatiles samples were analyzed using gas chromatography/mass spectrometry (GC/MS) to 1) identify the volatile compounds' composition ("compound richness"); and 2) determine the amount of volatiles released ("volatiles abundance") in dung of various ages. The GC/MS analyses were carried out using the automatic thermal desorption (TD) system (TD-20, Shimadzu, Kyoto, Japan) coupled to a Shimadzu GCMS-QP2010 Ultra equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m, i.d. 0.25 mm, film thickness 0.25 µm, Phenomenex). For more details on GC/MS and column settings see Heiduk et al (2016). The samples were run with a 1:1 split and a constant helium carrier gas flow of 1.5 ml/min. The GC oven temperature started at 40°C, then increased by 6°C/min to 250°C and held for 1 min. The MS interface worked at 250°C. Mass spectra were taken at 70 eV (EI mode) from m/z 30 to 350. GC/MS data were processed using the GCMSolution package, Version 2.72 (Shimadzu Corporation 2012). Identification of compounds was carried out using NIST 11, Wiley 9, FFNSC 2 as well as the database available in Mass-Finder 3 (Mitchell et al., 2015). To quantify the amount of each compound, known amounts of aliphatic, aromatic, and terpenoids were injected and the mean peak area was used for quantification (Dotterl et al., 2006). The amount of volatiles released per pat was standardized to ng.min^{-1} for further analyses.

Insect Sampling

Both beetles and flies were sampled from artificially created dung pats of 1.5 litres volume. Such artificially created dung pats were also created from stalled cows' dung, as the pats for volatiles sampling were. Following (Barth et al., 1994), we assume that although there might be some small differences in beetles and flies preferences for dung from pasturing cows and silage/hay fed cows (used in our study). Those difference however do not create two unique communities (Barth et al., 1994). From perspective of potential attraction between insect and dung volatiles along dung pats' ageing, succession on both such dung types should follow the very same pattern, indicating similar or identical volatiles emission (cf. Lee & Wall, 2006; Sladecek et al., 2013; Sladecek et al., 2017b).

Beetles were sampled during five sampling sessions in 2009 (11 – 29 April, 17 May – 4 June, 4 – 22 July, 15 August – 2 September and 23 September – 11 October), flies were sampled during three sampling sessions in 2011 (23 April – 1 May, 16 – 24 July, 26 August – 3 September) and three sampling sessions in 2012 (9 – 17 May, 27 July – 4 August, 14 – 22 September). Beetle adults and larvae were sampled by floating the dung pat and the underlying portion of soil in a bucket of water. Adult flies were sampled from the dung pats' surface by help of a sweeping net. Both beetles and flies were collected from 1, 2, 3, 5 and 7 day old dung pats, flies additionally from 1 hour old pats (each successional time was replicated five times in each season). There were virtually no beetles at this successional point while flies reach their highest abundance on 1 hour or less aged pats (Hammer, 1941; Mohr, 1943; Sladecek et al., 2017b). For additional details on insect sampling, see (Sladecek et al., 2013; Sladecek et al., 2017b).

Statistical analyses

1) Is there a succession of dung volatile compounds along the dung pat's ageing?

Number and amount of compounds released from pats of different ages were analyzed by Generalized linear models with mixed effects (GLMM) in R 2.15.2 (Team, 2012) using "glmer" function in package lme4 (Maechler & Bolker, 2011). Different models were used for analyses of number and amount of compounds. While for both tests dung pat age was used as a predictor and replication (to which of those five pats used for a successional time in a specific season the sample belongs) as a random factor, the model used to analyze the number of compounds was fitted with Poisson distribution of errors, whereas the model to analyze the amount of compounds was fitted with Gamma distribution of errors with log as a link function.

The succession of dung volatile compounds was analyzed by Detrended Canonical Correspondence Analysis (DCCA) with detrending by second order polynomial using CANOCO 5 for Windows (Ter Braak & Smilauer, 2012). DCCA is a multivariate technique suitable for datasets with response variables' unimodal response along environmental gradients (Leps & Smilauer, 2003). In addition, it also eliminates the arch effect artifact (which is presented in volatile data in non-detrended analysis). The amounts of volatiles were log (x+1) transformed prior to the analysis. Dung pat age was used as environmental variable with affinity to replication used as a block covariate. Statistical significance was assessed by Monte Carlo permutation test (999 permutations). After such analysis, we used Ward's hierarchical clustering in R 2.15.2 (Team, 2012) to identify successional groups of compounds. This clustering was performed using the species position on the "successional" DCCA axis (the first canonical axis).

2) Are there correlations between the abundance and species richness of dung-inhabiting insects and the amount and number of volatiles during ageing of dung pats?

To test for linkage between insect (beetle and fly) abundances / species richness and amount / number of volatiles, we performed eight different GLMMs in R 2.15.2. (Team, 2012). Every insect variable (e.g. beetle abundance) was tested for linkage with the number and abundance of volatiles using the volatile traits as predictor variables. For each successional time in insect data, we used mean volatiles' amount and number for that particular successional time from our chemical data. This was done because we have one seasonal session of chemical data and five seasonal sessions of insects data. We assume that the dung volatiles' succession does not change substantially between years and seasons (as in carrion (Perrault et al., 2015; Stadler et al., 2015)). In addition to chemical traits as main predictors, dung pat age, seasonal session and affinity to a replication (all taken from insect data) were used as factors with random effects in our GLMMs. All such models were fitted using the "glmer" function in package lme4 (Maechler & Bolker, 2011) and Poisson distribution of error was employed in all those models.

3) Is there trend between the composition of the insect fauna and the volatile patterns released during succession?

Beetle and fly species' succession was analyzed with DCCA in CANOCO 5 for Windows (Ter Braak & Smilauer, 2012). Separate DCCAs were fitted for beetles and flies' species with successional time as a environmental variable and season and affinity to a replication as block covariates. Statistical significance was assessed by Monte Carlo permutation test (999 permutation). Patterns of beetles and flies were then compared to DCCA patterns of volatiles analyzed in 1). Insects' reaction to individual compounds were not tested since dung-inhabiting insects, at least beetles, seems to be attracted to an assemblage of compounds, rather than a single one (Wurmitzer et al., 2017).

Results

We recorded a total of 54 dung volatile compounds, with major representation of terpenoids (4), nitrogen compounds (4), alcohols (5), and phenols (7). We were not able to fully identified 24 compounds (Table S1). All such compounds were used in analyses of volatiles trends and together with fly data. By exclusion of 1 hour old pats, for analyses of beetle trends, the compounds data contained 46 compounds. We collected a total of 28,284 beetle individuals from 86 species (five families) and 5,139 fly individuals from 37 species and morpho species (17 families).

1) Is there a succession of dung volatile compounds along the dung pat's ageing?

Both per pat volatiles amount ($t= -3.208$, $p = 0.00134$) and number ($t=-3.89$, $p < 10^{-6}$) were generally negatively correlated with the dung pat age. The volatiles amount decreased from 1 hour old pats to 2 days old pats. After that, there was however a huge peak of volatiles abundance in 3 days old dung pats followed with relatively stable, yet low volatiles abundances in 5 and 7 days old pats (Fig 1). The number of volatiles decreased more steadily with its lowest values in 2 days old dung, while the number of volatiles was almost identical in 1 and 3 days old pats (Fig 1).

Dung volatile compounds displayed a significant successional trend ($F = 14.6$, $p = 0.001$, first axis explains 35.5 % of variability, which is 89% of explainable variability). The Ward's clustering along the successional axis resulted into 2 major clusters: the 1) early-successional compounds that occurred until 2 days of dung pat age; and the 2) late-successional compounds that occurred after the 2 days of dung pat age (Fig 2, Fig S1). Samples from early successional stages were dominated by aliphatic and aromatic alcohols, such as 1-butanol, phenol, and p-cresol. Some of the compounds, such as 3-Methylbutanal, were even found only in samples collected from pats of 1 h or 1 day old. Dung of late successional stages released mainly nitrogen- (e.g. 2,6-Dimethylpiridine) and sulfur-bearing compounds (Dimethyl trisulfide), and aromatic esters (Isobutyl benzoate). Such stages also released unknown compounds in high relative amounts (UNK 1365 and 1378).

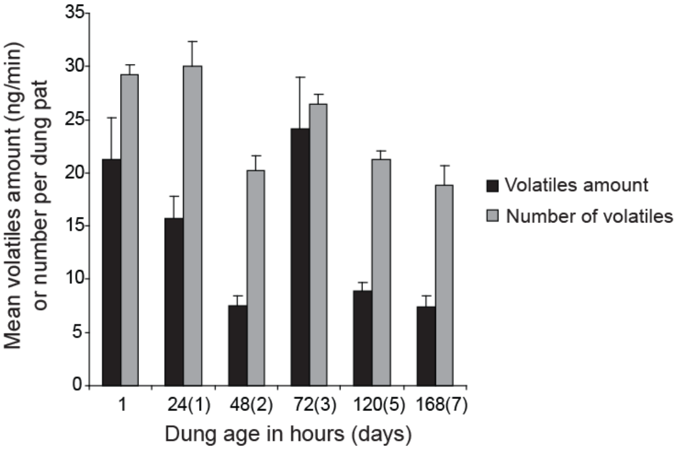

Fig 1: Mean amount and number of volatile compounds released from dung pats of different age.
Error bars represent standard error of mean.

2) Are there correlations between the abundance and species richness of dung-inhabiting insects and the amount and number of volatiles during ageing of dung pats?

Fly abundance (t = 2.806 , p = 0.005) and number of species per pat (t = 1.993, p = 0.046), were positively significantly associated with number of volatile compounds (Table 1). Otherwise there was no significant association between insect and volatiles, although beetle abundance was marginally significantly (p = 0.098) association with number of compounds (Table 1).

Table 1: GLMMs results of insect abundances and species richness relation to the volatile amounts and number.
(+) = positive significant association

	beetle abundance	beetle species richness	fly abundance	fly species richness
volatiles'	t = -0.747	t = 0.023	t = -0.8377	t = -0.488
amount	p = 0.455	p = 0.981	p = 0.402	p = 0.626
volatiles'	t = 1.654	t = 1.027	t = 2.806	t = 1.993
number	p = 0.098	p = 0.305	p = 0.005(+)	p = 0.046(+)

3) Is there trend between the composition of the insect fauna and the volatile patterns released during succession?
Both beetle (F = 13.3, p = 0.001, first axis explains 9.9% of variability, which is 61% of explainable variability) and fly communities (F = 7.4, p = 0.001, first axis explains 4.5% of variability, which is 50% of explainable variability) were significantly affected by dung pats' ageing.

The most striking result is, that the vast majority of species of both insect groups reached their optima together with either early successional group of compounds (flies, optima till 2 days old dung) or with late successional group of compounds (beetles, optima in 2 day old dung and older) (Fig 2). There were only very few species deviating from this pattern (8 fly species and morpho species, e.g. Chironomidae species; 3 beetle species, e.g. the dung burying *Aphodius erraticus*).

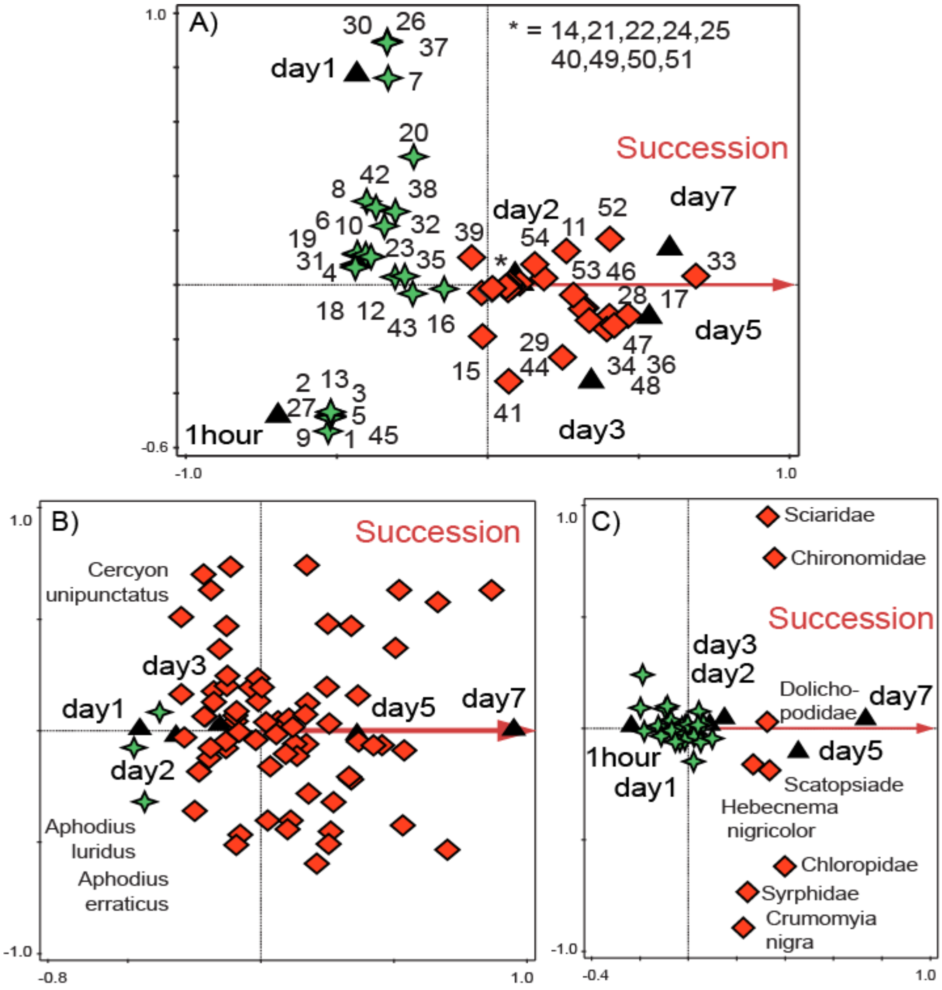

Fig 2: DCCA ordinations of volatiles and insects collected from dung pats of different age.

Individual successional points (1 hour, day1, etc.) are plotted as supplementary variables (up-triangles). Species symbols represent affinity to early (up to 48 hours old dung; green stars) or late (dung older than 48 hours; red diamonds) successional group of dung emitted volatiles.

A) Succession of dung emitted volatiles, separation between early and late successional groups is based upon clustering in Fig S1. For volatiles' abbreviations see Table S1

B) Succession of dung-inhabiting adult beetles. Species names represent three species having their optima along with early successional volatiles (green stars).
C) Succession of adult dung-inhabiting Diptera. Species names represent species having their optima along with late successional volatiles (red diamonds).

Discussion:
Based upon DCCA and clustering, we have found two prime successional groups of dung emitted volatile compounds. The early successional group (with optima until 48 hours old dung) consisted primarily of aliphatic and aromatic alcohols, while the late successional group consisted primarily of nitrogen- and sulfur-bearing compounds, and aromatic esters. The abundance and species richness of flies, but not of beetles, were positively associated with the number of compounds released during aging of the pats. At level of individual species, beetle species' successional optima overlapped predominantly with optima of late successional volatile compounds (dung older than 48 hours). In contrast, successional optima of vast majority of fly species did overlapped with optima of early successional compounds (up to 48 hours old dung).

Succession of dung-emitted volatiles
The temporal segregation of early and late successional compounds could have several explanations. The early successional compounds could be a result of bovine anaerobic fermentative digestion, in which alcohols are by-product of sugar assimilation (Boumba et al., 2008; Dekeirsschieter et al., 2009; Forbes & Perrault, 2014), or a result of protein degradation (phenols) (Dekeirsschieter et al., 2009), presumably of dead excreted stomach symbionts and epithelial cells. In addition, both volatiles abundance and compound richness declined throughout the early successional phase (till 48 hours old dung), which could support gradual loss of these early successional compounds that were originally delivered by the defecating cows. Another explanation could be based upon low

aeration of dung pats in this successional phase which enabled further anaerobic fermentation by bovine defecated symbionts (Boumba et al., 2008).

The late successional compounds on the other hand could be a result of insect activity. This activity is either direct, pheromones and metabolite emissions (e.g. Burger et al., 1995; Burger & Petersen, 2002), or indirect since insects significantly affect the development of bacterial (or microbial) and fungal communities (Blackwell & Malloch, 1991; Lussenhop et al., 1980; Stevenson & Dindal, 1987). The direct insect contribution to the pool of late successional compounds is highly possible, yet only anecdotal evidences are available. Pheromones of dung-inhabiting insects are relatively understudied now, however there is some evidence that they could structurally belong among late successional compounds, like derivates of carboxylic acids (Burger & Petersen, 2002). Other insect metabolites, namely the products of defensive glands, might also contribute to the late successional compounds, e.g. some terpenoids in Staphylindae beetles (Dettner & Schwinger, 1982; Huth & Dettner, 1990) and also in some Scarabaeidae beetles (Cortez et al., 2012). Additionally, fly larvae in carrion were found to emit basically the whole spectrum of carrion volatiles, that is characteristic for the successional stage when the larvae occur in large quantities (Frederickx et al., 2012a). This result could, however, be an artifact of methodology as authors themselves admit (Frederickx et al., 2012a). Finally, the nitrogen- and sulfur- bearing compounds could be a result of nucleotide and protein degradation of insects killed by predators (Dekeirsschieter et al., 2009). Indirect effect of insect activity on volatile compounds could be attributed to insect burrowing activity in dung pats. When a dung pat is created, it is a very wet mass with little to none gas exchange between outside and majority of its volume. This is later altered heavily by insect activity which both helps to aerate the dung (Skidmore, 1991), creating ever widening galleries under the dung crust (Skidmore, 1991). Thus they provide more air and therefore induce interchange between alcohol emitting anaerobic microbes and aerobic microbes (Boumba et al., 2008;

Lussenhop et al., 1980), and also facilitating the emission of nitrogen- and sulfur- bearing compounds due to degradation of the early successional bacteria (Dekeirsschieter et al., 2009).

Patterns of insect succession insight the successional patterns of dung-emitted volatile compounds

Contrary to the "clear" separation of beetle and fly species between early and late successional compounds, insect abundance and species richness were generally not correlated with volatile compounds' abundance and richness. This is probably caused by the "non-linearity" of dung compounds succession, in which the most prominent was the interchange between the early and late successional groups. The significant correlation between compound richness and fly dynamics could be rather attributable to fly association with early successional compounds; where both fly and early successional volatile compounds decreased sharply form 1 hour old dung towards 3 days old dung (Sladecek et al., 2017b).

The main result of our study is, however, how the two most prominent dung-inhabiting insect groups reflected the separation between early and late successional compounds (with few exceptions). As dung-inhabiting insect should fully depend on chemical cues when locating their food (Dormont et al., 2010; Halffter & Matthews, 1966; Wurmitzer et al., 2017), preference for either early or late successional compounds could signal the insects that either the environmental conditions are suitable for their activity (habitat filtering) (Keddy, 1992; Kraft et al., 2015), that they avoid inter-specific competition (niche differentiation) (Silvertown, 2004), or there is a high number of prey available at the pats (Hulcr et al., 2006; Segura et al., 2012).

The habitat filtering scenario appears to be the most probable, at least in temperate communities, where beetles co-occur with flies, i.e. their larvae, in the succession (Sladecek et al., 2017a). In that case, the early successional compounds would tell adult flies that the dung is fresh, without serious crust (personal observation), and available for their adult feeding on dung fluids or for ovipositing (Erzinclioglu, 1996; Hammer, 1941). The late successional compounds could signal to adult flies that there is a hard crust on the dung pat with dung-empty galleries under it (unsuitable for oviposition) (Skidmore, 1991). Additionally, such galleries host ever increasing community of beetle predators feeding on adult flies (Sladecek et al., 2013). For beetles, the early successional compounds could signal high levels of dung moisture which could be lethal for beetles (Whipple et al., 2013).

Utilization of dung-emitted volatile compounds to promote niche differentiation could rather apply in communities with presence of dung relocating beetles (e.g. tropics) which are able to destroy the dung pat in relatively short time (Horgan, 2002; Krell-Westerwalbesloh et al., 2004). In such communities, early successional compounds could be a sign of only chance to lay eggs or adult feeding for flies, since the dung pat could be away in matter of minutes or hours. Late successional compounds could signal non-relocating beetles (functionally similar to temperate beetle community) that relocating beetles already left the dung pat and it is therefore safe to oviposit there (Davis, 1996).

Attraction of dung-inhabiting predators into dung pats has never been rigorously studied. However, in many habitats, including rotting fruit (Segura et al., 2012) or rotting wood (Hulcr et al., 2006), predators do infiltrate the habits based upon either prey-emitted volatiles or volatiles associated with habitat degradation. Thus, it is possible to speculate that predator's invasion of dung pat could be a reaction to either massing of their potential preys in late succession (and rather to chemical impulses produced by them) or it could be a fixed preference for dung-emitted volatile compounds which signal availability of their food.

Although the insects' segregation between early and late successional compounds was quite significant, there are some exceptions from this pattern. In both beetles and flies, species deviating form the general pattern are either habitat generalists (flies, e.g. Chironomidae species) (Hammer, 1941) or avoid otherwise harsh conditions of fresh dung pats (soil-ovipositing beetles, e.g. *Aphodius erraticus*) (Gittings & Giller, 1997; Holt & Huxel, 2007). Both such exceptions should support the habitat filtering, as flies who avoid very wet habitats were associated with late successional compounds (like majority of beetles), while somehow moisture tolerant beetles were associated with early successional compounds (like majority of flies).

Comparison of dung and carrion

Contrary to carrion, dung should emit the highest abundance and compound richness of its volatiles at the very early stage of its ageing, while carrion is emitting the highest amount (probably) and compound richness of volatiles during mid to mid-late phase of its ageing (Dekeirsschieter et al., 2009; Paczkowski et al., 2015; Stadler et al., 2015). Those different dynamics is attributable to the difference between dung and carrion decomposition. Dung pats are usually covered with crust, between 1-3 days age, that is getting increasingly hard with insect activity (Skidmore, 1991). Although the crust does not prevent volatiles emission (Laubach et al., 2013), the emission is generally lower than in dung with weak or none crust (this study). Carrion on the other hand should emit increasing amount of volatiles with insect activity, as insects burrow through it, destroying it and enlarging its surface (Matuszewski et al., 2011; Sharanowski et al., 2008; Tabor et al., 2004). In contrast to dung, there are more successional stages of volatile compounds along carrion ageing, however as long as there are conflicting results (very early and very late successional compounds), it is impossible to draw any more detailed comparison with dung dynamics in our study (Dekeirsschieter et al., 2009; Paczkowski et al., 2015; Stadler et al., 2015).

Finally, due to strong successional pattern of all beetle and fly species, there seems to be no compound preference among individual functional groups (i.e. saprophages, predators, etc.) of beetles and flies (Sladecek et al., 2017a). In contrast, there is probably some separation between ecological groups in carrion insect community, as for example competitively dominant (Suzuki, 2000) carrion burying beetles are attracted to fresh to mid-successional carrion by oligosulfids (Kalinova et al., 2009; Podskalska et al., 2009; von Hoermann et al., 2013), while the competitively inferior carrion dwelling hide beetles are attracted to very dry late successional carrion by carboxylic derivates (von Hoermann et al., 2011).

Directions for future studies

Our study was to our best knowledge the first study comparing development of volatile compounds and insect/other animal community along gradient of habitat ageing. From this point onward, we would encourage the future studies to deal with the following topics: 1) Volatiles and insect dynamics in different dung types (e.g. horse, sheep) to support our results retrieved from cow dung, but more importantly in dung types created from different diet (herbivore x omnivore), since omnivores' dung seems to be chemically in-between herbivore dung and carrion (Stavert et al., 2014); 2) Volatiles and insect dynamics in communities with dominant dung relocating beetles (tropical, Mediterranean), to examine the potential niche differentiation based on dung volatiles; 3) Influence of insects and influence of other dung-inhabiting biota, to test which dung volatiles are produced or inducted by insects and which are result of other biota activity; 4) Insect responses to the volatiles, to identify the specific compounds responsible for attraction of flies and beetles; 5) Predators attraction, to test if predators are attracted by their prey metabolites or by general dung volatiles.

Acknowledgement

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. This work was supported by the Institute of Entomology, Biology Centre of the Czech Academy of Science (RVO//:60077344), by the Grant Agency of University of South Bohemia (152/2016/P). STS acknowledges funding from and a Univ. of South Bohemia Postdoc project (reg.no. CZ.1.07/2.3.00/30.0006) and the Grant Agency of the Czech Republic (grant number 15-24571S).

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Supporting information

Table S1: Dung emitted volatile compounds sampled in our study.
UNK XY = unidentified compound with retention index of XY, Abb. = numerical abbreviation used in ordination diagram, Suc. = early (optima until 48 hours old dung) or late successional group (optima in dung older than 48 hours), Ret. time = retention time, Ret. index = retention index, Total amount = total amount of particular volatile compound sampled (ng/min).

Compound name	Abb.	Chemical class	Suc.	Ret. time	Ret. index	Total amount
3-Methylbutanal	1	aldehyde	early	5,722	667	2.136
1-Butanol	2	alcohol	early	5,800	670	3.171
Pentanal	3	aldehyde	early	6,385	698	1.216
3-Methyl-1-butanol	4	alcohol	early	7,100	731	1.817
2-Methyl-1-butanol	5	alcohol	early	7,186	735	1.739
1-Pentanol	6	alcohol	early	7,800	763	6.092
2,3-Hexanedione	7	ketone	early	8,188	781	1.955
UNK 807	8		early	8,775	807	2.208
Isopropyl butyrate	9	ester	early	9,642	840	0.686
UNK 859	10		early	10,140	859	4.022
2,6-Dimethylpyridine	11	nitrogen compound	late	10,800	884	8.307
2-Heptanone	12	ketone	early	10,958	890	5.245
2,4-Dithiapentane	13	sulfur compound	early	11,050	894	2.348
alpha-Citronellene	14	terpenoid	late	12,085	932	10.188
3-Ethylpyridine	15	nitrogen compound	late	12,920	962	3.132
1-Heptanol	16	alcohol	early	13,090	968	8.346
Dimethyl trisulfide	17	sulfur compound	late	13,363	977	6.030
Phenol	18	phenol	early	13,371	980	14.950
UNK 1007	19		early	14,165	1007	5.914
UNK 1011	20		early	14,270	1011	2.120
UNK 1041	21		late	15,073	1041	10.414
UNK 1056	22		late	15,462	1056	13.225
p-Cresol	23	phenol	early	15,950	1074	54.410
UNK 1079	24		late	16,103	1079	10.493
UNK 1088	25		late	16,340	1088	9.371
UNK 1094	26		early	16,517	1094	1.254
2-Methoxyphenol	27	phenol	early	16,517	1095	1.675
UNK 1117	28		late	17,106	1117	5.926
UNK 1127	29		late	17,342	1127	9.208
N-(3-Methylbutyl) acetamide	30	nitrogen compound	early	17,403	1130	1.274
4-Ethylphenol	31	phenol	early	18,365	1166	11.099

3-ethyl-Phenol	32	phenol	early	18,410	1168	2.794
UNK 1179	33		late	18,671	1179	3.404
UNK 1185	34		late	18,830	1185	6.769
Terpinen-4-ol	35	terpenoid	early	18,905	1188	6.521
2-Pentylpyridine	36	nitrogen compound	late	19,283	1202	6.186
UNK 1209	37		early	19,450	1209	1.157
UNK 1219	38		early	19,680	1219	5.809
UNK 1227	39		late	19,860	1227	8.671
b-Cyclocitral	40	terpenoid	late	20,005	1232	11.837
UNK 2027	41		late	20,277	1243	2.694
cf. 3-Propylphenol	42	phenol	early	20,670	1260	2.337
4-Ethylguaiacol	43	phenol	early	21,320	1287	4.375
UNK 1293	44		late	21,477	1293	2.278
Indole	45	aromatic heterocyclic	early	21,770	1304	1.139
Isobutyl benzoate	46	ester	late	21,920	1312	6.700
UNK 1365	47		late	23,120	1365	70.441
UNK 1378	48		late	23,419	1378	10.495
UNK 1408	49		late	24,090	1408	10.462
UNK 1413	50		late	24,190	1413	10.730
Dihydro-beta-ionone	51	ionon	late	25,030	1452	10.875
beta-Selinene	52	terpenoid	late	26,290	1512	2.797
UNK 1518	53		late	26,427	1518	7.773
UNK 1789	54		late	31,627	1789	8.119

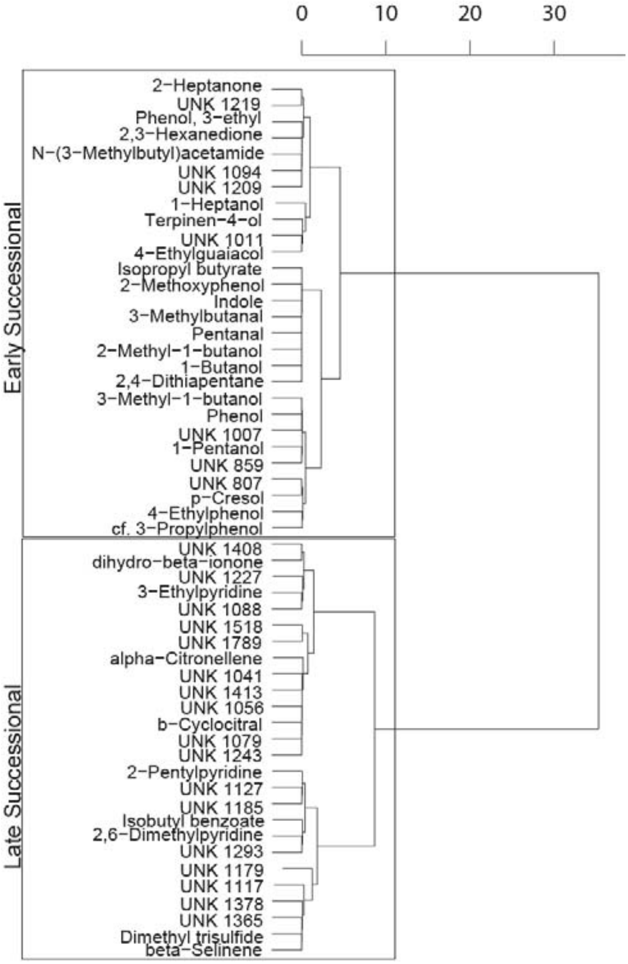

Fig. S1: Hierarchical clustering of dung volatile compounds along the gradient of dung pat ageing.

The clustering is based upon dung emitted volatile compounds' successional optima and conducted by Ward's hierarchical clustering in R 2.15.2. This clustering of early and late successional compounds is used throughout the study.

SUMMARY

This thesis brings new results that should be of an interest to all three fields of ecology on which the thesis was focused on, the dung habitat researchers, the ephemeral habitats researchers and general ecologists. For better resolution, I will therefore split the summary into three such respective parts.

In the narrow field of dung ecology, this thesis is novel mainly in presenting ecological data regarding dung-inhabiting dipteran species, both adults and larvae. The quantitative data on succession and seasonality of dipteran adults (CHAPTER I) are completely novel, as studies including dipteran adults did so mostly qualitatively or semi-quantitatively (Hammer 1941; Mohr 1943). Despite the species' overall patterns are similar, I have shown that succession of dipteran adults does not commence in a matter of minutes or hours as was thought earlier (Hammer 1941). I have also presented the fine-scale seasonal optima that could be vital for coexistence of species co-occurring during the succession. Perhaps even more important finding stems from combining ecological data on dipteran larvae with the rather well known picture of ecology of adult dung-inhabiting beetles (CHAPTER II) (Hanski & Koskela 1977; Gittings & Giller 1998; Lee & Wall 2006; Sladecek *et al.* 2013). As the majority of studies to date focused on adult beetles, the potential role of dipteran species was treated rather speculatively in the past (Hanski 1980; Sladecek *et al.* 2013). I have shown that despite competition might occur between beetle adults and dipteran larvae along successional gradient, this competition could also be reduced by seasonality. On the other hand, seasonal segregation of beetle and dipteran species seems to be driven rather by species temperature tolerances than by actual competition. The dung-inhabiting predators were mostly neglected ecological group as the major focus was given to coexistence of saprophages in former studies (Holter 1982; Gittings & Giller 1997; Sladecek *et al.* 2013). I have shown that the ecology of dung-inhabiting predators could be as complex as ecology of saprophages with their size-based separation along successional gradient and niche-based separation along seasonal gradient (CHAPTER III). Finally, the role of dung emitted volatile compounds was always highly regarded as attractants of insects to dung pats (Dormont, Epinat & Lumaret 2004; Dormont *et al.* 2007; Dormont *et al.* 2010). I have, however, shown that the whole succession of dung-inhabiting beetles and dipteran species could be structured by succession of dung emitted volatile compounds (CHAPTER IV).

In field of ephemeral habitats' general ecology, I have found both similarities and dissimilarities between dung and other ephemeral habitats (mainly carrion and rotting fungi). The patterns of successional and seasonal development of dipteran adults' communities are highly similar in both dung and carrion. In both communities, succession is characterized by decline of dipteran adults, which is more rapid on dung and less rapid on larger carrion (Matuszewski *et al.* 2011; Castro *et al.* 2012). Also, both dung and carrion inhabiting adult Diptera have a similar affiliation to higher temperatures and reach their peaks during summer months (Castro *et al.* 2012; Martin-Vega & Baz 2013). The temperature based-patterns of beetle and Diptera segregation along seasonal gradient present in dung resemble, to some extend, the segregation of carrion dipteran species from carrion relocating beetles, as carrion inhabiting beetles are generally also limited by high temperatures (Nisimura, Kon & Numata 2002). Finally, like in dung, members of carrion fauna display certain affinity to succession-based patterns of carrion emitted volatile compounds (Kalinova *et al.* 2009; von Hoermann *et al.* 2011; Dekeirsschieter *et al.* 2013; von Hoermann *et al.* 2013), although the succession of carrion volatile compounds seems to be more complex and has more stages than succession of volatile compounds in dung (Dekeirsschieter *et al.* 2009; Forbes & Perrault 2014; Paczkowski *et al.* 2015).

In field of general ecology, the dung community seems to follow the same principles of interactions between habitat filtering and niche differentiation retrieved from studies of other natural communities. Specifically, habitat filtering seems to apply between larger phylogenetic groups (Wiescher, Pearce-Duvet & Feener 2012; Mouchet *et al.* 2013; Arellano *et al.* 2016; Davison *et al.* 2016), i.e., seasonal segregation and succession based on dung emitted volatile compounds of beetles and Diptera in our study. Niche differentiation seems to apply among members of such larger groups (Adams & Thibault 2006; de Camargo *et al.* 2016), i.e., dipteran adults or individual predatory species in our study. In addition, succession of dung-inhabiting species displays patterns of solely habitat filtering. The successional patterns of dipteran adults, adult beetles and dipteran larvae, and dung-inhabiting predators should thus be driven entirely by dung pats' abiotic conditions rather than species niche differentiation as was suggested earlier (Hanski & Koskela 1977; Sladecek *et al.* 2013).

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APPENDIX

CURRICULUM VITAE
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Born: 17th of November 1987 in Havířov, Czech Republic
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1. Scientific background:

Education:

- Bc. (2007-2010): University of South Bohemia in Ceske Budejovice, Faculty of Science.
- Bc. Thesis: "Succession of dung beetle communities",
- MSc. (2010-2013): University of South Bohemia in Ceske Budejovice, Faculty of Science.
- MSc. Thesis: "Heterotrophic succession of dung insect communities of the warmer part of European temperate region".
- RNDr. (2013): RNDr. Thesis: "Interplay of succession and seasonality reflects resource utilization in an ephemeral habitat".
- PhD. (since 2013, hopefully till 2017): PhD. Thesis: "Community ecology of insects inhabiting ephemeral habitats".

Employment:

- Institute of Entomology of the Czech Academy of Science (since 2013): employed as a PhD student.
- Faculty of Science, University of South Bohemia (since 2014): Guarantor and sole lecturer of "Statistics of preclinical and clinical research I (KME 062) and II (KME 063)".
- Supervisor of 1 Bc. and 1 MSc. and co-supervisor of PhD Thesis (T. Zítek).

Research interests:

- Community ecology of insects inhabiting ephemeral habitats (especially dung and carrion): species coexistence (resource partitioning in time, space), food webs, environmental factors affecting such communities (chemical ecology, temperature tolerance).

- Taxonomy of Scarabaeidae: Scarabaeinae, since 2009 external specialist on Scarabaeinae:Onitini, Oniticellini for Entomological department of National museum in Prague.

2. Skills:

Languages:

- Czech (native speaker).
- English (fluent both in written and spoken).
- German (pre-intermediate in spoken, beginner in written).
- Able to work with taxonomic literature in French, Spanish (and other languages).

Statistical and other computer-related skills:

- Basic (STATISTICA, R) and advance (R) univariate statistics (including GLM, LME, GLMM).
- Multivariate statistics (CANOCO 5, R).
- Food web statistics (R).
- Basics of phylogenetics statistics (PAUP, MrBayes).
- Data visualization (R, Microsoft Excel, Adobe Illustrator).
- Able to process GC-MS chromatograms.

Taxonomy skills:

- Coleoptera -> Scarabaeidae, Hydrophilidae, Staphylinidae, Histeridae
- Diptera -> Muscidae, Calliphoridae, Sphaeroceridae, Sepsidae

Lab skills:

Basics of DNA/RNA extraction, PCR, DNA cloning.

3. Collaborations, conferences and publications:

Collaborations:

- Dr. Stefan Dötterl, University of Salzburg, project topic: Does succession of dung-inhabiting insects reflects changes in dung volatiles along dung pats' ageing?
- Prof. Clarke Scholtz, Dr. Adrian Davis, University of Pretoria, project topic: Temporal trends and competitive dominance in tropical and temperate dung-inhabiting insect communities.
- Prof. Richard Wall, University of Bristol, project topic: Temporal patterns among temperate dung-inhabiting beetles and flies.
- Dr. Petr Šípek and his NecroTeam, Charles University in Prague, project topic: Resource partitioning (in time, habitat type), food webs and chemical ecology of insect communities inhabiting small carcasses (and potential comparison with dung-inhabiting communities).

Conferences:

- Zoodny: several talks (2011,2013,2016)
- ENTO' 16 (2016), Royal Entomological Society, UK:
    - How do species rich communities of dung-inhabiting beetles and flies coexist? Through temporal segregation (talk given by me).
    - Temporal segregation among carrion insects; how species' succession and seasonality interact with carrion type (co-author on poster).

Publications:

- Bezdek A, **Sladecek F** (2012) *Platyonitis oberthueri* Janssens, 1942 and *Epionitis tarsatus* Balthasar, 1942 (Coleoptera: Scarabaeidae: Scarabaeinae: Onitini) — synonymy confirmed. Zootaxa **3267**: 65-68.
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In preparation:

- **Sladecek FXJ,** Dötterl S, Schäffler I, Simon ST, Konvicka M. Succession of dung-inhabiting beetles and flies reflects the succession of dung-emitted volatile compounds.

- **Sladecek FXJ**, Zitek T, Segar ST, Konvicka M. How do the temporal trends of dung-inhabiting predators affect their coexistence?
- **Sladecek FXJ**, Segar ST, Konvicka M. Early successional colonizers both facilitate and inhibit the late successional colonizers in communities of dung-inhabiting insects.
- Zitek T, **Sladecek FXJ**, Segar ST. Habitat size separates the co-occurring potential competitors in an ephemeral habitat.

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Community ecology of insects inhabiting ephemeral habitats.
Ph.D. Thesis Series, 2017, No. 18

Printed in the Czech Republic by Typodesign
Edition of 20 copies

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