Insect effects on bacteria and fungi in cattle dung

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Insect-microbial interactions were studied during the first month of dung decomposition in Wyoming and Michigan. At both sites, screen cones placed over fresh dung were used to exclude insect colonists and confine normal field densities of Aphodius beetle adults or enough sarcophagid adults to produce normal larval densities. The effect of these insects on bacterial and hyphal densities as well as on fungal species numbers was assayed after the dung had been in the field 3 to 4 wk. Presence of maggots and Aphodius beetles increased bacterial and decreased hyphal density in Wyoming but not in Michigan. We hypothesize that these effects are due to insect mixing of the substrate, giving bacteria a competitive advantage over fungi. Normal insect colonization increased the number of fungal species per pat in Michigan and Wyoming, although the total number of fungal species was not affected by treatments.

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Взаимодействия насекомых и микроорганизмов исследовали в течение первого месяца разложения навоза в Вайоминге и Мичигане. В обоих местах, на поверхности свежего навоза помещали экранирующие конусы, которые препятствовали колонизации насекомыми и ограничивали нормальную плотность их личинок Aphodius либо достаточную плотность взрослых муко-саркопагов, обеспечивая нормальную плотность личиночной популяции. Изучали влияние этих насекомых на плотность бактерий и грибных гиф и на эквивалентное их количество после того, как навоз находился в полевых условиях в течение 3–4 недель. Присутствие мух или жуков Aphodius увеличивало плотность бактерий и снижало плотность грибных гиф в Вайоминге, но не в Мичигане. Мы предполагаем, что это – результат перемешивания насекомыми субстрата, что дает бактериям преимущество в конкуренции с грибами. Нормальное загрязнение навозом увеличивает количество видов грибов в Мичигане и в Вайоминге, хотя общее количество видов грибов не зависит от внешних факторов.

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Introduction

The effect of invertebrate activity on microbial metabolism in decomposing substrates is believed to enhance decomposition of all but the "softest" tissues (Edwards et al. 1970). However, the basis for the stimulus, the specific effects of invertebrates on particular microbial groups, are poorly known (Satchell 1974). When is grazing and when is substrate mixing the important effect? Do invertebrates which stimulate bacterial metabolism also stimulate fungal metabolism? How are fungal species numbers affected by invertebrates? These questions motivated this study of insect-microbial interactions during the first month of decomposition of cattle dung in an arid short-grass range in Wyoming and a mesic pasture in Michigan. At both sites we contrasted dung with and without insects in order to show how their activity affected bacterial and fungal densities as well as fungal species composition.

We chose cattle dung for an examination of insect-microbial interactions because rapid development of bacterial, fungal, and arthropod populations might be expected to result in strong interactions. Among the first colonists are insects whose mouthparts allow them to concentrate fine particles from moist dung. Cyclorrhaphous fly larvae, such as species in the sarcophagid genus Ravinia used in the present study, sieve particles 0.6 μm or more from dung between pharyngeal ridges (Dowding 1967). Adult Aphodius beetles concentrate particles by pressing dung between labial and labral pads of setae (Madle 1934, Landin 1961). At the same time insects arrive, spores of coprophilous fungi, stimulated to germinate by gut passage through cattle, are believed to begin development of hyphal networks.

Materials and methods

Dung was obtained from pastured cattle so that the fungal inoculum they consumed would be fresh and representative of the site and season. The experiments were conducted near Laramie, Wyoming (Univ. of Wyoming Experimental Farm) from 29 July to 26 August, and in southeastern Michigan (Kellogg Biological Station, Michigan State Univ.) from 13 August to 3 September 1977. Adult Herefords were used in Wyoming; 12 to 16 month old Guernseys were used in Michigan. Dung was collected in the field of Wyoming, while in Michigan dung was collected from cows temporarily confined in a barn, and was frozen three days until use. We will not make between-site comparisons of microbial densities and species numbers because of the freezing. However, microbial densities in frozen and unfrozen dung incubated in the field for 3 wk in July 1977 and June 1978 did not show a response to freezing. At both sites 1 kg pats were immediately placed inside wire screen cones. The screen cones (0.6 mesh mm"·") were lined with muslin (3 mesh mm"·") and sand was placed around the bases of the cones to exclude arthropods.

The five treatments were: (1) presence of maggots belonging to early colonizing fly genera (in Wyoming, only Ravinia species were used; in Michigan half or more of the individuals were Ravinia, the remainder, species of Orthelidia and Musca), (2) presence of early beetle colonists of approximately equal size (Aphodius vitatus Say and A. scabriceps LeConte were used in Wyoming, A. haemorrhoidalis L. in Michigan), (3) presence of maggots and beetles, (4) no insects, (5) normal insect colonization. Each treatment was replicated five times in Wyoming and four times in Michigan. Wire screen cones were placed over dung subjected to each treatment; treatments were effected by adding unsexed adults as indicated in Tab. 1. In the case of normal insect colonization, treatment 5, open topped cones were raised about 15 cm above the ground on wooden blocks. Two dung pats were placed under cones to which adult insects were added, and the reproductive success of added adults was assayed by assuming that the number of arthropods extracted from one of the pats under each cone was a reasonable estimate of arthropods in the other.

Comparisons were made with arthropod densities in cohorts of dung pats put out in the same pastures on 1 August 1977 in Wyoming and Michigan; these are termed unmanipulated in contrast to dung manipulated with use of cones.

Dung was exposed in the field for 28 d in Wyoming and 21 d in Michigan. These intervals were chosen to allow for sporulation of the earlier appearing coprophilous fungi during field exposure at either site. Within 30 min of collection, manipulated dung pats were divided and returned to the laboratory: arthropods were extracted from one half in Berlese funnels; one quarter was frozen for later microbial biomass estimates; one quarter was frozen until each could be incubated for 40 d to allow sporulation of fungi which in the field might have sporulated during the next period of suitable moisture and temperature. Percent moisture was determined from frozen samples. Water potential of dung was estimated on the basis of a pF - % moisture relationship determined using filter paper of known matric potential (Fawcett and Collis-George 1967).

Microbial biomass was determined in the 3-4 mm thick crust as well as in the center of each dung pat. Fungal biomass was determined by counting hyphae in dung-agar suspensions, and bacterial biomass by counting bacteria stained with fluorescein isothiocyanate (FITC). Frozen 2 g dung samples were dispersed for 2 min in a Waring blender with enough sterile distilled water to make a 10⁻² dilution. Preparations for fungal counts were prepared by pipetting (pipette tip i.d. = 5 mm) 20 ml of the dispersed solution from the center of the column of the 10⁻² suspension and mixing with 30 ml of 2% purified agar solution. This mixture was used to prepare agar films in haemocytometer wells as de-
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and maggots in the response Aphodius 30 in manipulated and unmanipulated species (1971). Then, using phase contrast microscopy, the number of hyphal intersections with an ocular grid were counted and converted to a length estimate using the formula of Olson (1950). Preparations for bacterial counts were made by pipetting (pipette tip i.d. = 2.5 mm) 0.01 ml of the 10^2 solution onto microscope slides, heat fixing, and following the staining procedure of Babiuk and Paul (1970). The FITC-stained cells were then counted with dark-field illumination; globular cells above 2 microns in diameter were considered yeasts. Fungal species numbers were determined by identifying species which sporulated during a 40 d laboratory incubation at 24°C with optimal moisture.

We counted bacteria in 20 fields, and hyphae in 80 microscope fields per replicate. The F-max test indicated that statistical analysis should be performed on the square root of hyphal density, and on untransformed averages of bacterial numbers per field.

Results

The goal of our treatments was production of comparable numbers of both adult Aphodius and maggots in the dung at both sites. Tab. 1 shows that this was accomplished, although after a week there was much higher Aphodius reproduction in Wyoming than Michigan. Aphodius larvae are sedentary; we argue that they had much less effect on dung microorganisms than adult beetles (P > 0.10 for the correlations between density of Aphodius larvae and fungal or bacterial density in each of the 10 Wyoming pats with beetles).

The initial percent moisture of dung used was similar (81, and 80 in Michigan and Wyoming, respectively). Averaged over treatments, the % moisture of dung pats when collected in Michigan was 80 in contrast to 37 in Wyoming. The high percent moisture in Michigan was partly due to 28 mm rainfall during the two days prior to collection. In Wyoming only the dung crust dried sufficiently to limit microbial growth (a pF estimate of 4.5).

Arthropods had no effect on final moisture content in Michigan. In Wyoming, however, dung with lowest arthropod densities (the no-arthropod, and normal colonization treatments) contained 33% moisture after 28 d, significantly lower (p < 0.05; t-test) than the 40% moisture in dung to which arthropods were added. In spite of slightly higher water content associated with arthropod activity, rapid evaporation from Wyoming dung concentrates biological activity in a smaller volume. Independent field drying experiments in which dry and wet center dung was weighed separately suggest that after 3 d, at the end of maggot activity, 5%, and after 7 d, at the end of adult Aphodius activity, 20% of the volume of wet center dung has dried. The lower arthropod density in unmanipulated Wyoming pats relative to those in Michigan (Tab. 1) may be caused by this loss of volume.

The field site and crust-center contrasts were responsible for larger microbial density differences than treatments effected (Fig. 2). The high densities of bacteria and yeast in Michigan dung may be due to higher moisture levels. Different moisture levels at the two sites interacted with the microbial response to arthropod treatments, because the treatment effects were consistently strongest in the crust of Michigan dung and in the center of Wyoming dung. F-ratios of the 12 one-way analyses-of-variance performed on the data were always higher for crust in Michigan and for center in Wyoming; this pattern is evident in Fig. 1.

Arthropods affected microbial density more strongly in Wyoming than in Michigan (Tab. 2), and their effects were opposite at the two sites. In Wyoming, microbial density in dung crusts was unaffected by presence or absence of arthropods. In the center of Wyoming dung pats, presence of Aphodius alone increased bacterial density by 53% and maggots increased density by 43% relative to dung without insects. (Fig. 1). But in the presence of both Aphodius and maggots, bacterial numbers only increased 27%. Wyoming Aphodius treatments also show a similar, though statistically nonsignificant, trend in increased yeast density. Hyphal density, however, was decreased 40% by Aphodius.

In Michigan, the opposite microbial responses to arthropods were observed. Arthropods had no effect on microbial biomass in the center of Michigan dung. In
Fig. 1. Microbial response to arthropod addition to cow dung incubated in the field under cones. Treatments are: - (no arthropods), F (maggots), B (Aphodius beetles), B+F (maggots + Aphodius). C (cones raised to allow colonization). Letters indicate statistical differences based on one-sided 5% confidence intervals (Dunnell 1955) for no-arthropod vs. treatment contrasts.

Tab. 2. The average number of fungal species sporulating on 250 g portions of cattle dung in the field and during subsequent 40 d laboratory incubation. Normal colonization significantly increased species numbers in both Michigan and Wyoming (P<0.01; Kruskal-Wallis test).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wyoming</th>
<th>Michigan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal colonization</td>
<td>18.0</td>
<td>14.0</td>
</tr>
<tr>
<td>No arthropods</td>
<td>12.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Flies only</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Flies + Aphodius</td>
<td>11.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Aphodius only</td>
<td>11.0</td>
<td>11.5</td>
</tr>
</tbody>
</table>

the crust, presence of *Aphodius* increased hyphal density and maggots decreased bacterial numbers, but the significance of arthropod effects in the crust is not clear. There was no arthropod effect on yeast in Michigan dung.

Thirty-eight species of coprophilous fungi were identified during 40-d incubations of 250 g portions of manipulated dung. Eleven species occurred in both sites, 12 species only in Michigan, 16 only in Wyoming. In both sites, the most frequent species tended to be those which sporulated in the field or during the first 10 d of incubation. In Michigan, one unidentified species of *Coprinus* sporulated twice on all replicates: first in the field, then 20 d later on the same dung in the laboratory.

Many more fungi sporulated in the field in Michigan than in Wyoming, probably because of the difference in moisture between the two field sites. After a field incubation only three-quarters as long as in Wyoming, 53% of the Michigan fungi had sporulated in contrast to 11% of the Wyoming species. Arthropods did not affect the total number of sporulating fungal species per treatment: the range for all treatments was 13–17 in Michigan, and 16–22 in Wyoming. Neither did arthropod addition affect the rate of sporulation; in Wyoming, the reduction in hyphal density caused by *Aphodius* did not retard sporulation. After 20 d of laboratory incubation, the rate of sporulation was the same for all treatments and sites.

Arthropods were important in dispersing some coprophilous fungal species. This is shown by increased numbers of species sporulating on normally-colonized dung (Tab. 2), although it is not clear whether additional species or greater densities of arthropods caused the increase. No specific fungal species are responsible for the increase; species limited to normally colonized dung were infrequent, occurring only on one (*Podospora anserina* (Ces. in Rabenh.) Niessl in Michigan and a member of the Mucorales in Wyoming), two (*Saccharolus glaber* (Pers. ex Pers.) Lamb. Michigan), or three replicates (*Podospora curvula* DeBary, Wyoming). The increased species density due to normal colonization can be observed among fungi sporulating as soon as the tenth day of laboratory incubation suggesting that insect dispersed coprophilous fungi are not necessarily late appearing species, and that spores of some coprophilous species may be able to colonize dung directly as well as after consumption by herbivores.

The effect of arthropods on weight loss of dung is unknown. However, arthropods had strongly contrasting effects on dung texture which suggest that dung with no insects or maggots alone would not easily fragment. Michigan and Wyoming dung containing *Aphodius* (including the normal colonization treatment) were crumbly in contrast to the tough, papier-maché-like consistency of dung with maggots or no insects.

**Discussion**

The beetle and fly species used in the present study are early dung colonists, however, their few days of activity had a long-lasting effect on dung microbe populations which we sampled 2 to 3 wk later. That this early insect effect is long-lasting is demonstrated by the similarity between our results and a short-term laboratory experiment using similar species (*Aphodius rufipes* L., *A. fimetarius* L. adults; *Pareglea aestiva* Meig. maggots) in
sheep dung (Breymeyer et al. 1975). Breymeyer and her colleagues added ten times the insect densities of the present experiment, and quantified microbial abundance using agar plate cultures. They found, as in the Wyoming dung of the present study, that Aphodius adults decreased numbers of fungal propagules, while both maggots and Aphodius adults increased bacterial density.

Why was no microbial response observed in Michigan dung? An answer cannot be given because both climate and dung properties differ between sites. We speculate that more rapid drying of dung in Wyoming concentrates arthropod activity. Further, forage plants and thus dung texture differ resulting in flatter pats with a greater surface area in Wyoming; this might also concentrate arthropod activity. Finally, freezing the Michigan dung may have increased initial microbial growth. Nevertheless, existence of a different response at each site is important, because it demonstrates that very similar insect congeners may have contrasting effect on microorganisms in different habitats.

Why did bacterial and hyphal density respond differently to insects in Wyoming dung? One hypothesis is that adult Aphodius feeding destroys bacterivorous nematodes and their eggs. This does not explain the bacterial increase in response to maggots, which because of differently constructed mouthparts are unlikely to affect nematodes. A more consistent hypothesis is that in the presence of insects the competitive advantage of hyphal growth form is lost to fungi. With arthropod mixing, bacteria are continually exposed to fresh substrate, they do not deplete resources within diffusion distance of their cells and stop growing. In addition, nutrients in arthropod feces, and possibly lowered antibacterial effects of fungi may contribute to the effect. With arthropod mixing of dung, the ability to extend a hyphal network into fresh substrate is less advantageous to fungi because their growth rates are generally lower than those of bacteria.

Aquatic amphipods appear to increase bacterial productivity by mixing similarly to dung insects (Fenchel 1970, Harrison 1977). Hargrave's (1970) study of Hyalella azteca Saussure feeding on sediment cores from the benthos of lakes is especially pertinent because substrate disturbance by Hyalella azteca increased bacterial productivity, but, at high densities, decreased algal productivity.

The present study suggests that insects may reduce microenvironmental extremes in dung. In wet, possibly anaerobic Michigan dung, insects may aerate dung. In Wyoming, where insects were associated with higher % moisture, separation of a crust from moist dung underneath by arthropod activity may reduce capillary water movement between the wet dung center and the surface.

As dung ages and dries, insects emigrate or pupate, and hyphal density increases. The expansion of hyphal networks into the center of drying dung has been illustrated by Dickinson and Underhay (1977) who recorded densities similar to those observed in the present study. By using two field sites, we have shown that although fungal reproduction is not affected, hyphal density may be reduced by insects. Over a range of moisture conditions, insect activity may result in bacterial and fungial activity occurring sequentially under dry conditions, or simultaneously under moist conditions.

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References


Dowding, V. M. 1967. The function and ecological significance of the pharyngeal ridges occurring in the larvae of some cyclorrhaphous Diptera. - Parasitology 57: 371-388.


