ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 63 173 Number 5, 2015
http://dx.doi.org/10.11118/actaun201563051565

FOOD OFFER INSIDE AGROECOSYSTEM SOILS AS AN ECOLOGICAL FACTOR FOR SETTLING MICROHABITATS BY SOIL SAPROPHAGOUS MITES

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Abstract

SMRŽ JAROSLAV, KUČERA TOMÁŠ, VAŠKŮ ZDENĚK. 2015. Food Offer Inside Agroecosystem Soils as an Ecological Factor for Settling Microhabitats by Soil Saprophagous Mites. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(5): 1565–1574.

Mainly abiotic factors have been considered in examining soil fauna invasion or settlement. The role of soil animals communities was not considered. Our hypothesis, indeed, can be formulated: the structure and feeding habits of the soil animals community is not able to play some role in the soil rating. Localities, however, can be fragmented into microhabitats. We studied cultivated field and adjacent unploughed areas (so-called baulks), using the common Berlese–Tullgren apparatus for community structure studies followed by histological tests of food consumed by community members. We selected a group of oribatid mites, which are frequent and abundant. In the studied localities and their microhabitats, three groups of oribatid mites can be reported. First – ubiquitous species a second – migrants from the less-impacted to more-impacted microhabitats and third – specialists sensitive to severe environmental conditions in more-impacted microhabitat. They consequently live only in the less-severe, less-impacted unploughed soils and never migrate from these microhabitats. Their grazed and digested food is more diversified, and they included more feeding specialists.

Keywords: mites, community structures, food digested, microhabitats, migration

INTRODUCTION

Abiotic factors have been considered as governing and defining soil localities and their dwellers (Wallwork, 1976; Lavelle and Spain, 2001; Coleman *et al*., 2004). Increasing fluctuation of such factors naturally results in more severe conditions and, therefore, poorer animal communities. On the other hand, adaptations of animals make possible the settlement of even severe localities or microhabitats (Smrž, 2006a).

As true of such localities as reclamation areas (Dunger *et al*., 2001; Frouz, 2008), agricultural tracts, and especially cultivated fields, constitute very extreme localities. Their specific characteristics result from recurring early succession stages due to repetitive agricultural treatment (ploughing, harvesting, in some plots manuring or fertilization, etc.). These practices mainly impact the actual cultivated field, whereas the neighbouring untreated areas are affected only indirectly and not to the same extent (Sheals, 1956; Andrén and Lagerlőf, 1980; Rockett, 1986; Lagerlőf and Andrén, 1988; Benckiser, 1997; Abbott and Murphy, 2003).

Relationships between soil type and classification or other abiotic parameters and the soil-inhabiting animal community have been studied only meagrely (Rockett, 1986; Benckiser, 1997; Abbott and Murphy, 2003). In some countries, systematic soil rating has been practiced, such as in the Czech Republic (Němeček and Kozák, 2005) as a system of so-called "rating units". It has been constructed for agricultural as well as legal and administrative purposes. That system is based on soil, topographical and climatic parameters (Němeček and Kozák, 2005)

and the rating consist of 5 numerals. For example, a soil may be rated as "4.56.00". The "4" denotes one of 10 climatic regions in the Czech Republic, the "56" is one of 78 main soil units (based upon soil type, subtype, matrix and granularity), the first "0" refers to slope and soil exposure, and the final zero to soil profile and skeleton.

While such unit would be applied to a fairly large area, there could be differences in microhabitats even within that given locality. Moreover, biotic conditions are wholly unrepresented in that soil rating unit and soil evaluation generally does not consider the richness of fauna in the soil.

We therefore formulated the following research questions:

- Are there differences between communities of soil mites dwelling in localities differing by by different soil rating units?
- Are there differences between two partially identical microhabitats within a given agricultural locality, for example a cultivated field and the adjacent unploughed soils (baulks)?
- Are abiotic characteristics the only factors governing migration into and settlement within localities and microhabitats?

2. MATERIAL AND METHODS

Localities and Sampling

Three localities with similar altitude (from 210 to 400 m a.s.l.) and a distance of 30 km (Tab. I) from one another were chosen in the Czech Republic's Central Bohemia Region. Temperatures and precipitation amounts in the localities were thus very similar. All plots were located in a moderately hilly landscape, but differed by soil type and hence, by Czech soil rating unit. The three soil types can be characterized generally as fluvisol (Locality 1), brown soil (Locality 2), and cambisol (Locality 3). Each locality included two microhabitats: cultivated field and unploughed soil.

Cultivated field was sampled approximately 50 cm from the boundary between the field and adjacent unploughed soil. The fields were conventionally farmed (e.g. ploughed, seeded, harvested) but without any manuring or fertilization for the two years of study. All fields were sown to cereal grains, in all three plots autumn wheat.

Unploughed soils (baulks) were immediately adjacent to the cultivated fields. Samples were taken approximately 30 cm from the boundary with the adjacent field. The unploughed area differed especially by its richer diversity of vegetation (Tab. I) in comparison to the cultivated field.

One sampling unit consisted of a set of 3 soil cores from each microhabitat collected every three months during 1–1/2 years. The three cores sampled from an individual microhabitat were subsequently mixed and extracted by Berlese–Tullgren apparatus into Bouin-Dubosque-Brasil fluid modified for oribatids (Smrž, 1989). Mites of two suborders – Oribatida and Acaridida – were identified to species level, with the exception of most specimens of the genus *Oppia* (Oribatida). Those species were very similar to one another and, moreover, not so frequent (only at Locality 1). The 10% level in relative abundance of species was estimated as constituting *eudominance* (Tischler, 1976).

Numerical Analysis

Numerical classification of mites (cluster analysis, TWINSPAN) was made using the program PC-ORD v. 5 (McCune and Mefford, 2006). The mite species abundance data were log-transformed for all analyses.

Multivariate analysis was used to describe presumed interrelations between species and environmental factors (Legendre and Legendre, 1998). Constrained ordination was computed in Canoco for Windows v. 4.5 (ter Braak and Šmilauer, 2002). The particular variance of species data explained by the environmental variables was studied by direct gradient analysis. The most striking environmental gradient was the habitat. We therefore expected a linear response of species to environment and selected the method accordingly. For direct gradient analysis, centring by species and scaling by interspecies correlation were selected and standardization by species was chosen due to the species differences in quantity. The explanatory effects of particular environmental variables were evaluated using Monte Carlo permutation test by a stepwise procedure that selects variables with the best fit of species data. This procedure tests the significance of regression (F-statistics and probability of Type I error) under the null hypothesis that species data are independent

I: *Studied localities, their soils, vegetation, Czech soil rating units and GPS coordinates*

Locality	Soil classification	Dominant herbs in unploughed microhabitats	Czech rating units	GPS coordinates
1. Račice	fluvisol modal mesobasic on aluvium moderatwe heavy	poppy (*Papaver*),yarrow (*Achillea*), cranesbill (*Geranium*), common dandelion (*Taraxacum*), buttercup (*Ranunculus*)	4.56.00	50°1'28.255" 13°55'40.561"
2. Lidice	brown soil luvic on loess moderate heavy bottom in land depression	grasses (*Lolium, Dactylis, Poa*)	4.10.00	50°8"18.614" 14°11'35.438"
3. Kladno	cambisol modal eubasic with cambisol modal mesobasic on slate moderate heavy	grasses (*Lolium Dactylis, Poa*)	4.26.01	50°7'22.662" 14°4'17.475"

of the environmental variables. The number of permutations was arbitrarily set at 4,999. The significantly important environmental variables were visualized by ordination biplots in Cano Draw v. 4.12 (ter Braak and Šmilauer, 2002). Eigenvalues (λ) indicate the explanatory power of the axes and express their relative importance (Lepš and Šmilauer, 2003).

Histology

A histological method was utilized for food consumption analysis. The internal anatomy of oribatid or acaridid mites was studied rather scarcely (Woodring and Cook, 1962; Smrž, 1989). At least five specimens per each species and location were embedded in Paraplast (Polysciences, Germany, through Sigma-Aldrich, Prague, Czech Republic). They were then sectioned on a Leica 2155 rotation microtome (Leica, Brno, Czech Republic) at 5 µm thickness, stained using Masson's triple stain, and observed under an AX-70 Provis (Olympus C & S, Prague, Czech Republic) light microscope, which included using a Nomarski DIC prism.

Six microanatomical characteristics were examined (Smrž, 2002; Smrž and Čatská, 2010) to identify the digestion processes and digestibility of food:

1) gut content (to identify food types consumed and progressive stage of digestion through gut);
2) activity of gut walls, as measured by apocrine secretion of wall cells (to determine whether food is digested or not);
3) metabolite deposits as guanine crystals (to identify digestion);
4) nutrient deposits as glycogen granules (to identify palatable, digestible food);
5) free cells (haemocytes) between the internal organs and within gut walls (for transporting enzymes: Smrž, 2006b); and
6) internal bacterial extraintestinal bodies (showing production of certain enzymes).

II: *Mites found and their abbreviations as used in Figs. 2 and 3 ("j" at front of abbreviation indicates juveniles)*

Achipteria coleoptrata (Linnaeus)		Achi col
Ceratozetes gracilis (Michael)		Cera gra
Ceratozetes mediocris Berlese		Cera med
Ceratozetoides cisalpinus (Berlese)		Cera cis
Eupelops occultus (C. L. Koch)		Eupe occ
Eupelops occultus (C. L. Koch)	juvenile	**j**Eupe oc
Galumna elimata (C. L. Koch)		Galu eli
Galumna elimata (C. L. Koch)	juvenile	**j**Galu el
Gustavia fusifera (C. L. Koch)		Gust fus
Hypochthonius rufulus C. L. Koch		Hypo ruf
Liacarus coracinus (C. L. Koch)		Liac cor
Liebstadia similis (Michael)		Lieb sim
Metabelba pulverosa Strenzke		Meta pul
Metabelba pulverosa Strenzke	juvenile	**j**Meta pu
Nothrus anauniensis Canestrini et Fanzago		Noth ana
Nothrus anauniensis Canestrini et Fanzago	juvenile	jNoth an
Oppia sp.		Oppia
Protoribates capucinus Berlese		Prot cap
Punctoribates punctum (C. L. Koch)		Punc pun
Punctoribates punctum (C. L. Koch)	juvenile	**j**Punc pu
Scheloribates laevigatus (C. L. Koch)		Sche lae
Scheloribates laevigatus (C. L. Koch)	juvenile	**j**Sche la
Tectocepheus velatus (Michael)		Tect vel
Tectocepheus velatus (Michael)	juvenile	**j**Tect ve
Tyrophagus putrescentiae Schrank		Tyro put

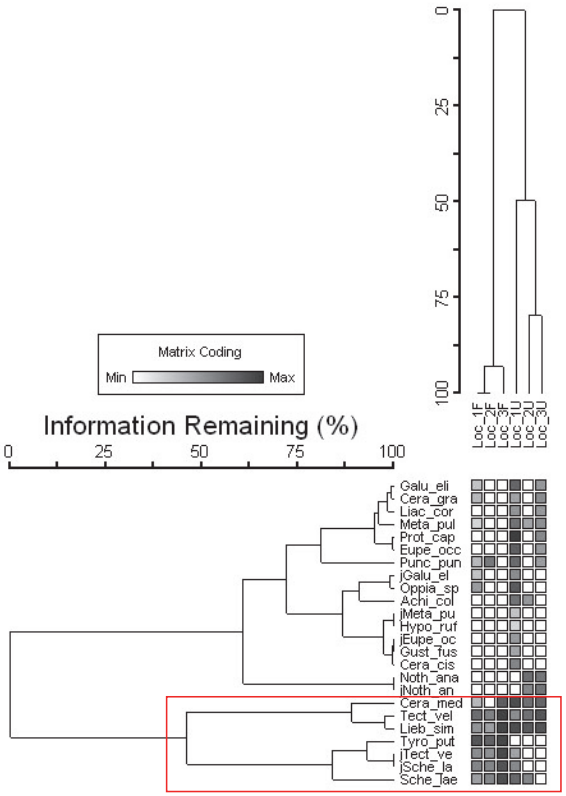
RESULTS

Mites and Community Structures

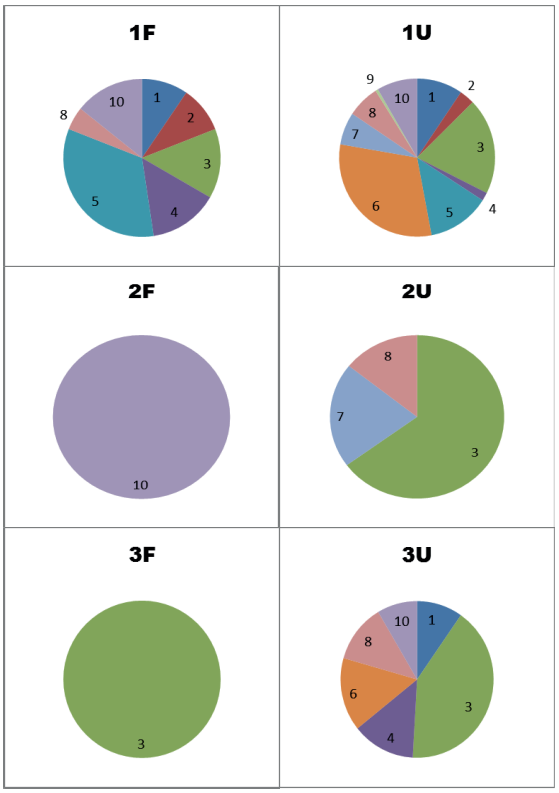
Altogether, 3,466 mites of 18 species were sampled (Tab. II). Species dominance seemed to be very important for the evaluation of localities. The study revealed differences not only among the three localities, but also within them (i.e. between their microhabitats). Three mite communities were established at all localities, and they can be

III: *Field mite species – abundances in cultivated field (CF) and unploughed soil (UP) (juv. = juveniles)*

Taxon	Localities and their microhabitats					
	1		2		3	
	CF	UP	CF	UP	CF	UP
Tyrophagus putrescentiae	103	0	74	0	166	0
Tectocepheus velatus	53	22	30	47	193	106
Tectocepheus velatus juv.	22	12	20	0	154	0
Scheloribates laevigatus	13	61	27	27	130	0
Scheloribates laevigatus juv.	27	24	27	0	116	0
Liebstadia similis	18	135	13	94	173	114
Total	**236**	**254**	**191**	**168**	**932**	**220**



Graph 1: *Two-way cluster analysis (Euclidean distance, Ward's method) of species and localities of 3,466 mites show the similarity of all 18 species found. The shaded squares represent the proportional species abundance. Full species names and abbreviations are in Tab. II*

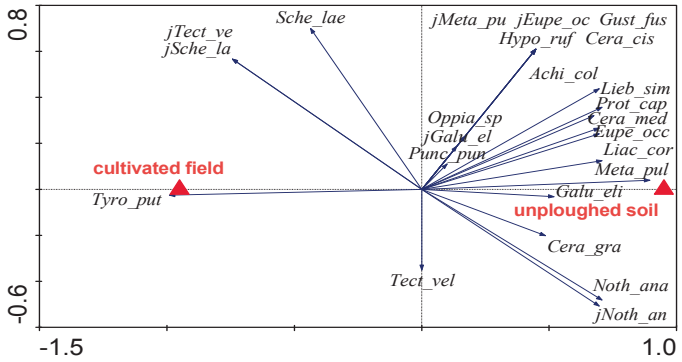


1 – *Galumna elimata*
2 – *Galumna elimata* juv.
3 – *Ceratozetes mediocris*
4 – *Ceratozetes gracilis*
5 – *Oppia* sp.
6 – *Protoribates capucinus*
7 – *Achipteria coleoptrata*
8 – *Metabelba pulverosa*
9 – *Metabelba pulverosa* juv.
10 – *Punctoribates punctum*

Graph 3: *Relative abundances of nomads in studied localities. Abbreviations used: F = cultivated field; U = unploughed soil*

characterized generally as field dwellers, nomads, and non-field dwellers.

Field dwellers (Tab. III) dominated in the cultivated fields. Their populations also included juveniles. With the exception of in fluvisol soil (Locality 1), they were less abundant in the unploughed microhabitats. *Tyrophagus putrescentiae* inhabited only field microhabitats. Two-way cluster analysis (using Euclidean distance, Ward's method) of species and localities showed the close ecological similarity of the aforementioned three species (Graph 1). The numerical analysis confirmed the close relationships of the field species, while at the same time highlighting a clear diversity in other groups.



Graph 2: *Direct gradient analysis biplot diagram shows on the first (λx = 0.43) and second (λy = 0.15) ordinal axes the species correlations along the environmental factor CF/US (fit 0.43, F-ratio 3.25, p-value 0.1); variance explained = 0.43, no. of permutations = 4999. The effect of locality was filtered out by covariates. Full species names and abbreviations are in Tab. II*

IV: *Mites migrating from unploughed soils (UP) into cultivated fields (CF) (nomads) – abundances in cultivated fields and unploughed soils (juv. = juveniles)*

Taxon	Numbers in Fig.3	Localities and their microhabitats					
		1		2		3	
		CF	UP	CF	UP	CF	UP
Galumna elimata	**1**	4	67	0	0	0	16
Galumna elimata juv.	**2**	4	22	0	0	0	0
Ceratozetes mediocris	**3**	6	139	0	50	85	68
Ceratozetes gracilis	**4**	6	12	0	0	0	22
Oppia sp.	**5**	14	91	0	0	0	0
Protoribates capucinus	**6**	0	216	0	0	0	25
Achipteria coleoptrata	**7**	0	47	0	16	0	0
Metabelba pulverosa	**8**	2	47	0	11	0	20
Metabelba pulverosa juv.	**9**	0	4	0	0	0	0
Punctoribates punctum	**10**	6	59	39	0	0	14
Total		**42**	**70**	**39**	**77**	**85**	**165**

V: *Mites from unploughed soils (baulks) only – abundances (juv. = juveniles)*

Taxon	Localities		
	1	2	3
Eupelops occultus	81	0	15
Eupelops occultus juv.	15	0	0
Liacarus coracinus	18	0	16
Gustavia fusifera	11	00	
Nothrus anauniensis	0	53	43
Nothrus anauniensis juv.	0	26	45
Ceratozetes cisalpinus	28	0	0
Hypochthonius rufulus	2	0	0
Total	**155**	**79**	**119**

A plot of the direct gradient analysis is shown in Graph 2.

Another ecological group of oribatid mites emerged in the microhabitats of all three localities. They migrated between the unploughed and cultivated microhabitats (*nomads*) (Tab. IV, Graph 3). The richest community once again occurred in the fluvisol of Locality 1, including its field microhabitat. That was in contrast to their sparse appearances in both microhabitats at the brown soil locality (Locality 2). *Ceratozetes mediocris* numbers were high in the unploughed microhabitat of Locality 3 (cambisol), and its abundance was even higher in the unploughed microhabitat of Locality 1 (fluvisol). On the other hand, juvenile mites wer completely absent in brown soil.

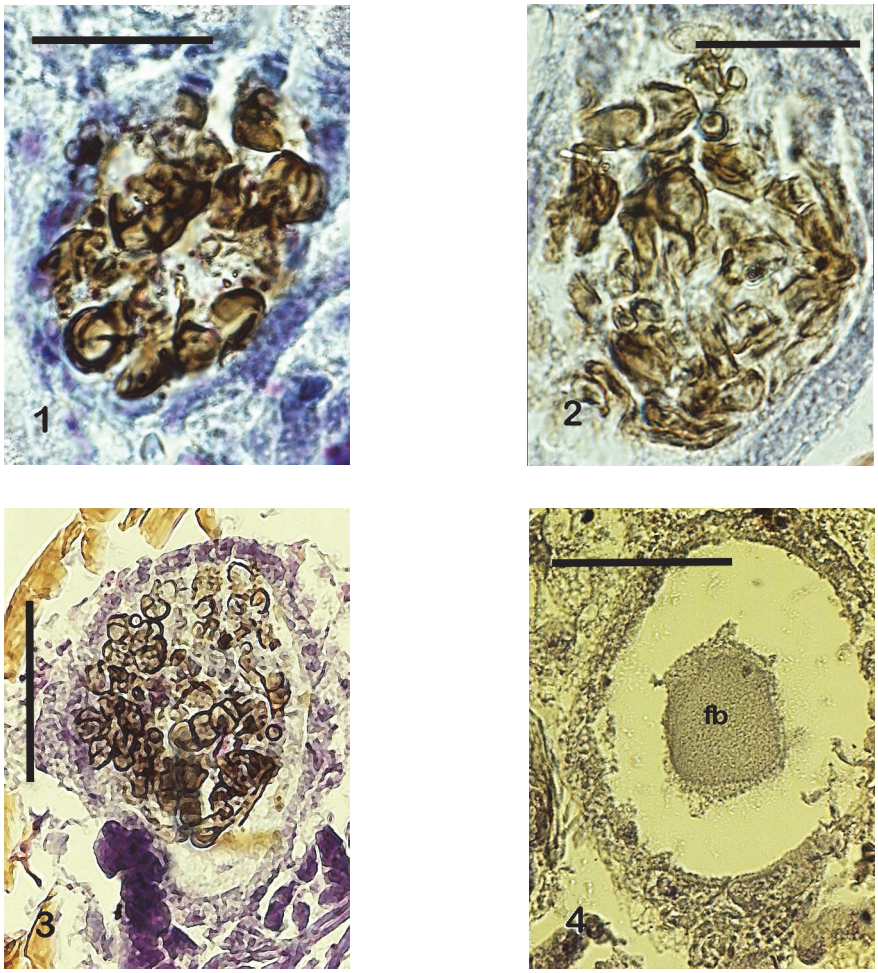
The third community of mites dwelled solely in unploughed microhabitats (Tab. V). *Nothrus anauniensis* never inhabited Locality 1 (fluvisol). The unploughed microhabitat of locality1 (fluvisol) hosted the most diverse community.

Food Consumption

In both microhabitat types, the field mites consumed mainly fungi, and mostly of a similar nature as judging by their propagules (Figs. 1–3, 5, 6, Tab. VI). Moreover, the field mites exhibited additional evidence as to the digestibility of the consumed food (metabolites and nutrient deposits) in both microhabitats. Only *Liebstadia similis* consumed only bacteria in both microhabitats (Fig. 4).

Migrating mites (nomads) consumed mostly bacteria in the field microhabitats, without any digestion-confirming phenomena (Fig. 7, Tab. VII). Their mesenteron looked empty and only lined by bacterial cover. In unploughed microhabitats, however, those nomads consumed mainly fungi with additional evidence as to the digestibility of the consumed food (Fig. 8). Only *Metabelba pulverosa* grazed fungi looking the same in both microhabitats and as digestible food (Fig. 9).

Dwellers of unploughed microhabitats consumed mainly fungi and manifested digestion-accompanying phenomena (Tab. VIII). The fungal propagules seemed to be of different natures in different mite species (Figs. 10–11). *Hypochthonius rufulus* formed a concetric bacterial bolus in the gut (Fig. 12).



1–4: *Alimentary tract, field: 1 – Tyrophagus putrescentiae, mesenteron with fungal spores 2 – Tectocepheus velatus, adult, mesenteron with fungal spores; 3 – Tectocepheus velatus, juvenile, mesenteron with fungal spores; 4 – Liebstadia similis, mesenteron, with bacterial bolus. Staining is by Masson's trichrome, DIC (4). Abbreviations used: co – colon, fb – food bolus, g – glycogen deposits, hem – hemocytes, me – mesenteron, re – rectum. Scale bars: 2μ (1–4)*

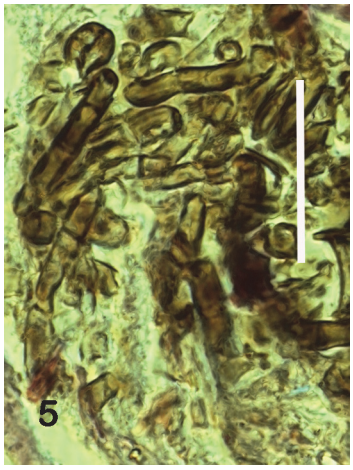
VI: *Food within gut and accompanying phenomena inside mites in cultivated fields (UP = unploughed soils, CF = cultivated fields, juv. = juveniles)*

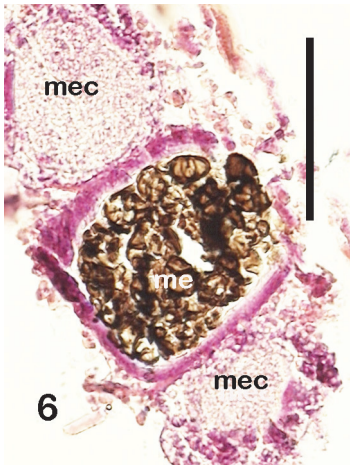
Species	Microhabitats in localities			
	CF		UP	
	food in gut	deposits and cells inside the body	food in gut	deposits and cells inside the body
Tyrophagus putrescentiae	fungi	haemocytes	fungi	haemocytes
Tectocepheus velatus	fungi, bacteria	haemocytes, guanin	fungi	haemocytes, glycogen
Tect.velatus juv.	fungi, bacteria	haemocytes	fungi	haemocytes
Scheloribates laevigatus	fungi, bacteria	haemocytes	fungi	haemocytes
Sch.laevigatus juv.	fungi, bacteria	haemocytes	fungi	haemocytes
Liebstadia similis	bacteria	none	bacteria	haemocytes, glycogen

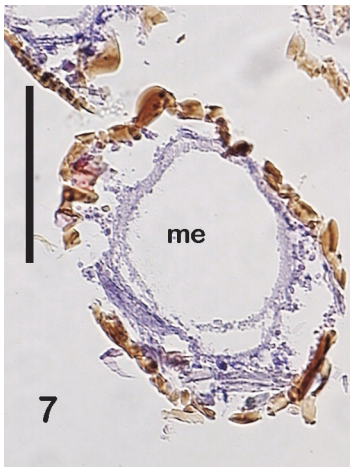
DISCUSSION

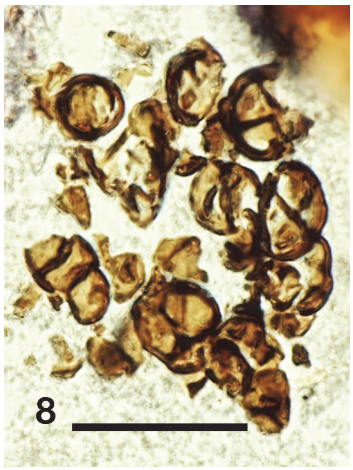
The diversity of agroecosystem animal communities is assumed to be very low, and without new additions. Nevertheless, these communities do play a biological role in fields, they vary between microhabitats, and their presence and activity may be used for characterizing the productive potential of soils and in rating soils for administrative purposes.

The field-dwelling species represented in this study comprise a homogeneous group with regard to their occurrence as well as food consumption, both of which were confirmed by numerical









5–8: *Alimentary tract: 5 – Scheloribates laevigatus, adult,mesenteron with fungal spores and fragments of mycelium, field; 6 – Scheloribates laevigatus, juvenile, field, mesenteron with fungal spores; 7 – Punctoribates punctum, mesenteron with bacterial lining, nomad, field; 8 – Punctoribates punctum, mesenteron, with fungal spores, nomad, unploughed microhabitat. Staining is by Masson's trichrome. Abbreviations used: me – mesenteron, mec – mesenteral caeca, re – rectum. Scale bars: 1 µ (6, 7), 2 µ (5, 8)*

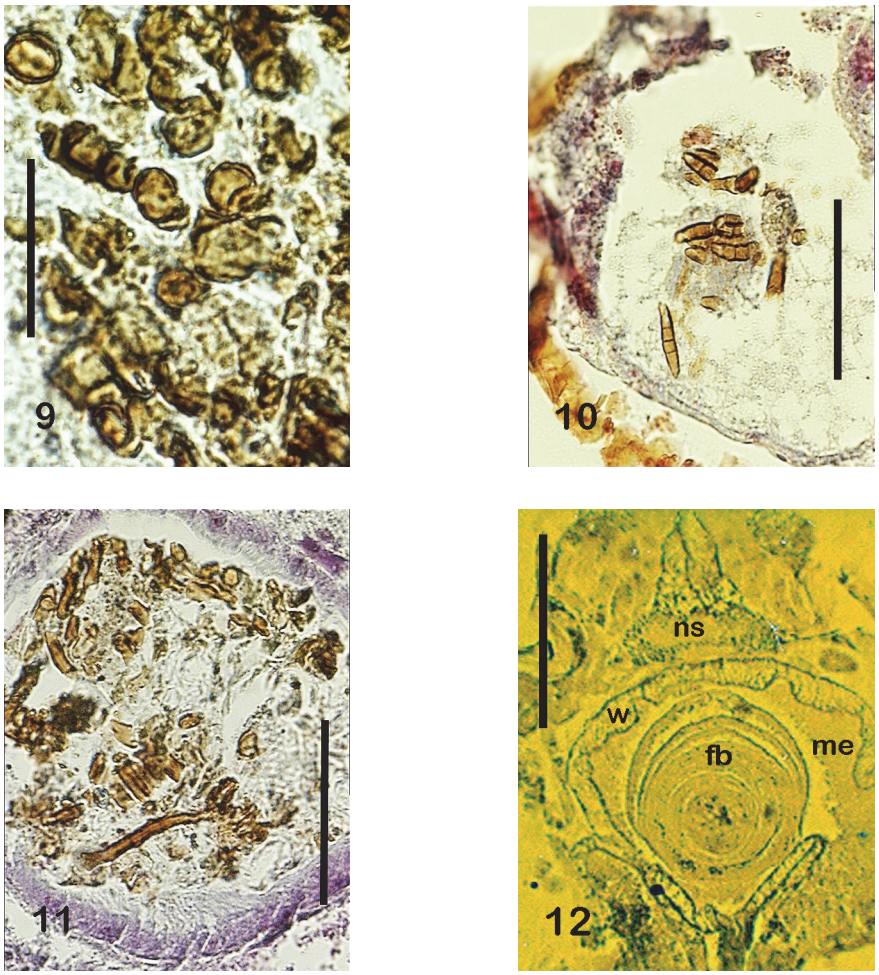
VII: *Food within gut and accompanying phenomena inside nomads (US = unploughed soils, CF = cultivated fields, juv. = juveniles)*

Species	Microhabitats in localities			
	CF		UP	
	food in gut	deposits inside body	food in gut	deposits inside body
Galumna elimata	bacteria	none	fungi	haemocytes
Galumna elimata juv.	bacteria	none	bacteria	none
Ceratozetes mediocris	bacteria	none	bacteria	haemocytes
Ceratozetes gracilis	bacteria	none	fungi	none
Oppia sp.	bacteria	none	bacteria	haemocytes
Achipteria coleoptrata	bacteria	none	fungi	haemocytes
Punctoribates punctum	bacteria	none	fungi	none
Metabelba pulverosa	fungi	haemocytes	fungi	heamocytes glycogen, IBEB
Metabelba pulverosa juv.	fungi	none	fungi	haemocytes

analysis. Such field communities were similar in all three localities without respect to different soil types or soil rating units. Their actual digestion of fungi was revealed by accompanying phenomena inside the mites' bodies from both cultivated field and unploughed microhabitats. The fungal propagules, however, resembled one another. The apparently very low number of fungi would confirm the low diversity of the fungal community (Čatská and Smrž, 1988) in agroecosystems. These

VIII: *Food within gut and accompanying phenomena inside mites from unploughed soils (juv. = juveniles)*

Species	food in gut	deposits and cells inside body
Eupelops occultus	fungi	haematocytes, IBEB +
Eupelops occultus juv.	fungi	haemocytes
Liacarus coracinus	fungi	IBEB
Gustavia fusifer	bacteria	haemocytes
Nothrus anauniensis	bacteria	none
Ceratozetes cisalpinus	fungi	haemocytes
Hypochthonius rufulus	bacteria in concentric bolus	none



9–12: *Alimentary tract, mesenteron with fungal spores, unploughed microhabitat: 9 – Metabelba pulverosa,mesenteron with fungal spores, nomad, unploughed microhabitat; 10 – Eupelops occultus, mesenteron with fragment of mycelium, unploughed microhabitat; 11 – Liacarus coracinus, mesenteron with fungal spores and fragment of mycelium, unploughed microhabitat; 12 – Hypochthonius rufulus, mesenteron with bacterial concentric bolus, unploughed microhabitat. Staining is by Masson's trichrome, DIC (12). Abbreviations used: fb – food bolus, me – mesenteron; ns – nervous system. Scale bars: 2 µ (9), 5 µ (10–12)*

mite species probably dwelled in both types of microhabitats, consuming and digesting food there as well as reproducing in most cases, as confirmed by the presence of juveniles. All these species, including *Liebstadia similis*, probably tolerated the ecological as well as biological conditions. *Tyrophagus putrescentiae* seems to be very tolerant of very severe microhabitats in terms of both ecology and biology (Smrž and Jungová, 1989) even avoided the more diversified conditions (Smrž, 2000).

The very important group designated as migrants also comprised a well-established group. Those species are sufficiently resistant to the fluctuation of abiotic factors in the treated fields for such microhabitats to accommodate searching migrations by those species. They do not avoid them. Those

mites, however, visit those microhabitats only for a short time, without consuming digestible food (Tab. VII) and without reproducing (no juveniles). All those species seem to be mobile, searching in surrounding microhabitats (Tab. IV). Their smooth body surfaces (*Galumna, Ceratozetes, Protoribates, Achipteria, Punctoribates*) appear similar to *Trichoribates trimaculatus,* an actual migrant (nomad) in moss cover localities (Smrž, 2006a). Our agricultural migrants consumed bacteria in cultivated fields without manifesting any accompanying phenomena of digestibility. In the mesenteron, those bacteria resembled the lining of the gut. They do not form an actual food bolus or faecal pellet as in truly bacteriophagous mites (cf. *Punctoribates* in the field – Fig. 7 with Fig. 12). In the unploughed microhabitats, however, they consumed mainly fungi, less frequently bacteria, and both mostly with characters corresponding to digestible food as true of the dwellers of such microhabitats.

The unploughed microhabitats was characterized by more diversified communities, although the abiotic factors (moisture, temperature, climate) were the same as in the cultivated, adjacent fields. There nevertheless were substantial differences, as the vegetation was more diversified in unploughed microhabitats. As a result, the soil animal community was also more diversified. This is seen both in the mite community structure itself and in the increased range of food types (different fungi) in mite mesentera. The diversity of fungi is visible in Figs. 8–11. *Metabelba pulverosa* is reported to be an orthodox mycophagous mite as well as a mobile animal (Smrž and Trelová, 1995; Smrž, 2002), and therefore its role as a nomad can be understood. The actual bacteriophagous species such as *Gustavia fusifera* (Drobná, 1999) or *Hypochthonius rufulus* (Smrž, 1989) were found among that ecological group.

CONCLUSION

Differences between localities were conspicuous in this study, and especially with regard to the structures of communities and their food consumption in unploughed soils.

The feeding habits appear to be very important for the migration and especially dwelling of mite species in microhabitats. Those habits represent the ecological factor corresponding to food offer in the microhabitats.

The study described here can be usefully incorporated into a supporting methodology for soil rating systems and for soil evaluation.

Acknowledgement

This study was supported by Grant No. 526/07/0393 from the Grant Agency of the Czech Republic.

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