

Soil-inhabiting tardigrade community facing first years of canopy dieback in the spruce mountain forest, Šumava National Park, the Czech Republic

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Abstract

The study investigated the impact of canopy dieback caused by a bark beetle outbreak on soil tardigrade communities in a mountain spruce forest of the natural zone Smrčina in the Šumava National Park. We found no substantial effect on the tardigrade community in the first two years after the infestation. However, an increase in abundance and minor changes in species composition were apparent. These effects were statistically insignificant, possibly due to the limited number of samples evaluated after canopy dieback. More data from the following years would be ideal to increase number of post-dieback observations. Last but not least, the study highlighted the importance of sufficient sampling density to detect rare species and noted the potential conservation significance of the rarely observed species *Diphyscon* cf. *bidropion*.

Key words: bark beetle, water bears, microfauna

INTRODUCTION

Tardigrades are microscopic eight-legged animals (Fig. 1). In an active state they need to be surrounded by water film (NELSON et al. 2018). However, they can turn into an inactive state called anhydrobiosis in dry conditions (MØBJERG & NEVES 2021). Therefore, they inhabit not only water bodies, but also soil, the surface of plants, and other periodically wet habitats (NELSON et al. 2018). Tardigrades were mostly studied in moss cushions. Nevertheless, soil and leaf litter harbour ca. one quarter of animal global biodiversity and probably hides many undescribed tardigrade species (DECAËNS et al. 2006). Nowadays, tardigrades include 1 464 species, but tens of new species are described every year (DEGMA & GUIDETTI 2023). Therefore, we can expect that the number will increase even more when we look into understudied environments such as soil.

Up to date, most studies on soil-inhabiting tardigrades dig no deeper than the first few centimetres of soil (e.g. HALLAS & YEATES 1972, GUIDETTI et al. 1999, GUIL et al. 2013, GUIDETTI et al. 2024). Despite the fact that the number of tardigrades decreases with soil depth, it was documented that depending on the depth of the organic layer, tardigrades can live in significant numbers as deep as 40 cm (ABE 1993). Moreover, although some tardigrade

species inhabit more than one microhabitat (MEYER & HINTON 2007), species composition of soil and mosses differ to a wide extent (e.g. BERTOLANI & REBECCHI 1996, GUIDETTI et al. 1999, GUIDETTI & BERTOLANI 2001, NELSON et al. 2020, GUIDETTI et al. 2024). There are even species that can be found only in soil and some of them have special adaptations to live there – such as worm-like bodies or reduction of claws (e.g. BERTOLANI & BISEROV 1996, VECCHI et al. 2022, VINCENZI et al. 2024). Coniferous forests seem to harbour the highest abundances of tardigrades, having typically several tens of thousands of individuals per square metre, while other habitats bear much lower numbers (HUHTA & KOSKENNIEMI 1975, HUHTA 1976, SOHLENIUS 1979, PERSSON et al. 1980, ITO 1999, ITO & ABE 2001, HARADA & ITO 2006). For example, the density of tardigrades in beech forests is typically 1–12 000 ind/m² (HALLAS & YEATES 1972), and in pastures only 654 ind/m² (FLEEGER & HUMMON 1975). However, their distribution is rather patchy and variable in very small scales (e.g. HOHBERG 2006, MEYER 2006, BARTELS & NELSON 2007, DEGMA et al. 2011). In addition, several species in the community often occur in very small numbers (e.g. ZAWIERUCHA et al. 2015, GUIDETTI et al. 2024). Therefore, to understand the whole community structure and catch the rare species, fine-scale investigations with repeated sampling can be very useful in the description of local tardigrade community patterns.

Although tardigrades are resilient to variable stressors, they react to many changes in the environment. Their abundances were previously related to soil structure, and age, nematode density, moisture, litter type, and other factors. (e.g. HARADA & ITO 2006, HOHBERG 2006, GUIL & SÁNCHEZ-MORENO 2013, BINGEMER et al. 2020). Recently, a large part of the spruce

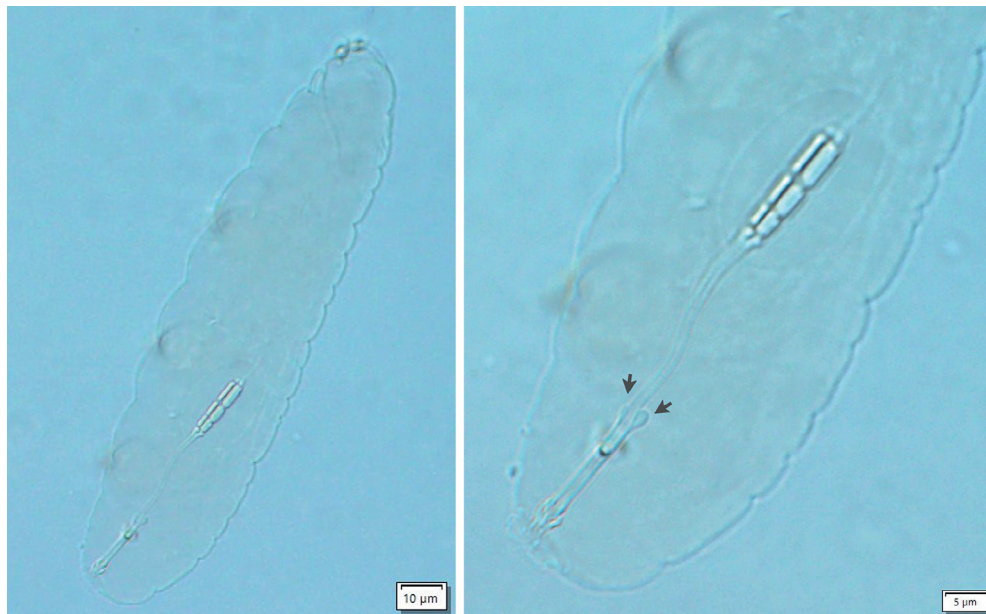


Fig. 1. *Diphascoen cf. bidropion* from mountain spruce forest in the Šumava National Park. Dark arrows point at the two apodemes, i.e. two drop-like cuticular thickenings between buccal and pharyngeal tube, which are characteristic for this species (photo: M. Tůmová).

forests in the Czech Republic was infested by bark beetles (HLÁSNY et al. 2021). Although global climate change probably promoted bark beetle infestation (HLÁSNY et al. 2011), the collapse of the tree canopy at higher elevations was presumably a result of a natural cyclical development of spruce forests with a final stage expressed by a large-scale dieback of the tree canopy due to bark beetle infestation (JANDA et al. 2014). It was shown that the short-term canopy dieback influences many soil properties, above-ground characteristics as well as animal communities (HAIS & KUČERA 2008, THORN et al. 2018). However, there is no such studies on tardigrades.

Tardigrades have similar life strategies as other microinvertebrates such as nematodes and rotifers. Despite their role in the ecosystem is supposed to be negligible due to their small biomass, tardigrades have been shown as potentially effective regulators of nematodes (HYVÖNEN & PERSSON 1996, HOHBERG & TRAUNSPURGER 2005). Moreover, their significance may grow after abrupt changes in conditions, since a massive increase in their abundance was documented after various disturbances (UHÍA & BRIONES 2002, HOHBERG 2006).

Existing studies on forest-inhabiting tardigrades are related to clear-cutting of trees in variably old plantations (HUHTA 1976, SOHLENIUS 1982, JÖNSSON 2003). However, clear-cutting and bark beetle outbreaks (without further human interventions) are two different disturbances when it comes to the effect on soil. Clear-cutting brings temperature and moisture fluctuations, while canopy dieback after bark beetle outbreak provides stable moisture and temperature conditions similar to those in the forest with live canopy (LINDENMAYER & NOSS 2006, HAIS & KUČERA 2008). Similarly, the only existing study on tardigrades after fire event (VICENTE et al. 2013) can be hardly compared with bark-beetle outbreak since increased temperature and loss of organic matter are limiting factors for survival of the organisms after fire disturbances (CERTINI et al. 2021).

Here we evaluate tardigrade abundance, species, and trophic composition in a mountain spruce forest in the natural zone of Šumava National Park and observe the changes in the soil tardigrade community during the first few years after the bark beetle outbreak.

MATERIAL AND METHODS

Locality, monitored plots, time schedule of the sampling

The research area is located on the slope of the Smrčina hill (1 195–1 245 m a.s.l.) in the natural zone of the Šumava National Park. Historically, the hill was used for pasture, then afforested by Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) and left without any intervention since 1995. Nowadays, the hill is overgrown by a mountain spruce forest with a relatively opened canopy (50–60% canopy cover), and *F. sylvatica* occurring in the canopy layer only rarely (only investigated plot P008 has beech in the canopy layer). Due to the low density of trees and relatively open canopy, the herbaceous layer has high coverage and is dominated by *Calamagrostis villosa* and *Vaccinium myrtillus*.

Ten square 20×20 m permanent plots were used for canopy dieback observation (Table 1). Soil for tardigrade extraction was sampled in three replications per plot; i.e. outside of the square plot near its upper left, upper right, and lower right corner when looking up the hill with the plot in front of a person. Samples were collected twice a year in June and October for three subsequent years. The sampling started in the spring of 2020 when the canopies of

Table 1. GPS coordinates of the centroids of the monitored plots in DMS format.

Plot	GPS coordinates in DMS format	
P001	48°44'33.834"N	13°55'41.469"E
P002	48°44'30.982"N	13°55'43.361"E
P003	48°44'27.968"N	13°55'47.209"E
P004	48°44'30.016"N	13°55'51.068"E
P005	48°44'28.683"N	13°55'54.054"E
P006	48°44'26.888"N	13°55'48.776"E
P007	48°44'26.014"N	13°55'52.498"E
P008	48°44'22.965"N	13°55'55.115"E
P009	48°44'22.188"N	13°55'52.124"E
P010	48°44'31.802"N	13°55'46.806"E

all investigated plots were still green and alive. During subsequent sampling events, the canopy was gradually dying as a result of bark beetle (*Ips typhographus*) infestation. We noted the state of the canopy dieback every sampling event and therefore, we could count the time from the beginning of the bark beetle outbreak at each plot. The canopy dieback throughout the years can be observed in the scheme Fig. 2. Eventually, we were able to observe not only a typical pattern of tardigrade communities in the mountain spruce forests, but also the immediate changes in these patterns caused by the bark beetle outbreak.

Collection and processing of the soil samples

Soil samples were taken with a metal cylindrical soil corer (diameter = 5 cm, depth = 10 cm), stored in plastic bags in the cold, and processed within one week after sampling. Each soil core was gently homogenized by hand and two subsamples of known weight were taken. The first subsample served for gravimetric measurement of dry soil. Soil was dried at room temperature until it achieves stable weight. Based on the ratio of original weight and dry weight of the subsample we calculated dry weight of soil used for tardigrade extraction. Later, we recalculated the numbers of extracted tardigrades not only per square meter as is usual in soil zoology (number of tardigrades per 1 g of dry soil \times dry weight of the whole soil core/area of the sampled soil core in m²), but also per a gram of dry substrate as is usual for tardigrade ecology (number of extracted tardigrades/dry weight of the subsample for tardigrade extraction) and, in addition, we extrapolated the numbers of extracted tardigrades per whole sample (number of extracted tardigrades per gram \times dry weight of the whole soil core). The second subsample served for extraction of tardigrades. Animals were extracted from the soil within 24 hours on the Baerman funnel modified by the addition of a high gradient of

temperature and light (CZERNEKOVÁ et al. 2018). The extracted tardigrades were fixed on permanent slides in Hoyer’s medium, counted, and identified to the species level following original descriptions listed in the 42nd Actual Checklist of Tardigrada Species (DEGMA & GUIDETTI 2023). The most relevant literature used for identifications was: RAMAZZOTTI & MAUCCI (1983), ITO (1995), GUIDETTI et al. (2009), PILATO & BINDA (2010), GAŚIOREK et al. (2016), VECCHI et al. (2016), KACZMAREK & MICHALCZYK (2017), GAŚIOREK et al. (2018), GAŚIOREK et al. (2019), TUMANOV (2020), GAŚIOREK & MICHALCZYK (2020), GAŚIOREK et al. (2023). Some of the species were identified only at the level of genus, groups of morphologically similar species (denoted as agg.), species with uncertain assignments (denoted as cf.), or potentially undescribed species with morphological affinity to the named species (denoted as aff.). For further identification, eggs or more individuals fixed in an ideal position to take morphometric measurements would be needed. Baermann funnel method, however, is based on the active movement of live individuals, therefore, it was impossible to extract the eggs.

Species were furthermore assigned into one of the three trophic groups – microbivores, herbivores, omni/carnivores – based on anatomical traits on bucco-pharyngeal apparatus (GUIDETTI et al. 2012, GUIL & SÁNCHEZ-MORENO 2013, ZAWIERUCHA et al. 2019) to evaluate changes in the role of tardigrades in the soil food web before and after canopy dieback.

Plot	Spring 2020	Autumn 2020	Spring 2021	Autumn 2021	Spring 2022	Autumn 2022
P001	live canopy	dieback	dieback	bark peels off	bark peels off	no bark
P002	live canopy	fresh dieback	fresh dieback	dieback	dieback	dieback
P003	live canopy	live canopy	live canopy	dieback	dieback	no bark
P004	live canopy	fresh dieback	dieback	bark peels off	bark peels off	no bark
P005	live canopy	live canopy	live canopy	fresh dieback	dieback	no bark
P006	live canopy	live canopy	fresh dieback	fresh dieback	dieback	no bark
P007	live canopy	live canopy	live canopy	live canopy	live canopy	live canopy
P008	live canopy	live canopy	live canopy	live canopy	live canopy	live canopy
P009	live canopy	live canopy	live canopy	live canopy	live canopy	fresh dieback
P010	live canopy	live canopy	live canopy	live canopy	live canopy	fresh dieback

Legend:

- live canopy all trees alive
- fresh dieback trees freshly infested by bark-beetle
- dieback trees dead, needles fell
- bark peel off trees dead, bark on the trunks started to peel off
- no bark trees dead, almost without bark

Fig. 2. Schematic depiction of canopy dieback observed from spring 2020 to autumn 2022 at the investigated plots in natural zone Smrčina.

Statistical evaluation

We evaluated abundance and Shannon diversity index in relation to time from the start of the canopy dieback, using Linear mixed effects models (LME), with Time from canopy dieback modelled as the explanatory variable with fixed effect, plot identity as a factor with random effect, and sampling event as another random effect factor to account for repeated measurements of the same plot. Abundance data were log-transformed to increase the normal distribution of the data. For LME fitting and calculation of the significance, lme4 (BATES et al. 2015) and lmerTest (KUZNETSOVA et al. 2017) packages within an R software (R CORE TEAM 2024) have been employed, respectively. Boxplot and connected point plots were constructed in R software using ggplot2 package (WICKHAM 2016).

Community composition was evaluated as changes in the proportion of individual taxa in relation to time from the canopy dieback. Species response curves were fitted by general additive models in CANOCO program (TER BRAAK & ŠMILAUER 2012).

Trophic group composition was evaluated in a similar fashion. We ran repeated measures CANOCO to evaluate differences in time accounting for the variability of individual plots. A stacked column bar plot was constructed in Microsoft Excel software (MICROSOFT CORPORATION 2018).

RESULTS

General patterns in tardigrade community

Typical abundance of tardigrades throughout the three years was on average 2–5 ind/g dry soil (10^4 ind/m²). Average as well as maximum numbers of tardigrades change rather annually than seasonally. Overall abundance shows an increase from spring 2020 to spring 2022 (Table 2), which is probably related to the dieback of tree canopy (see the next section). At the last sampling event in autumn 2022, the number decreased.

During the three years, we have found a total of 23 tardigrade taxa (Table 3). The number of taxa observed throughout individual sampling events was variable and ranged between 9 and 17. Till the sampling event in autumn 2022, we had always recorded some new, previously not observed taxa with every new sampling. Dominant species throughout the seasons were microbivorous *Mesocrista revelata* and *Adropion scoticum*. In some of the sampling events other taxa such as microbivorous *Diphascon pingue* agg. (spring 2020) and *Astatumen trinacriae* (from spring 2021 to autumn 2022), or omnivorous *Macrobotus hufelandi* agg. (autumn 2020, spring 2021, spring 2022) represented a dominant part of a community. Herbivorous tardigrades such as *Dianeia* sp., *Hypsibius* sp., or *Rammazzottius* sp. constituted, in total, always at max. 5% of tardigrade communities.

Many taxa (*Milnesium* sp., *Diphascon* cf. *bidropion*, *Diphascon* cf. *higginsii*, *Diphascon nobilei* agg., *Adropion belgicae*, *Notahypsibius* sp., *Hypsibius* aff. *scabropygus*, *Paramacrobotus richtersi* agg.) were found only rarely, i.e. only several sampling events and in small numbers. Some taxa were observed only in single samples (*Dactylobiotus* sp., *Hypsibius* cf. *exemplaris*, *Isohypsibius* sp., *Rammazzottius* sp.).

Table 2. Overall average, minimum, and maximum abundances (in ind.g⁻¹, ind.m⁻², and ind .soil core) and the number of observed species during individual sampling events. Legend: S = spring, A = autumn. One sample includes cylindrical soil core with the volume 196 cm³.

Observed variable	S2020	A2020	S2021	A2021	S2022	A2022
Average (ind.g ⁻¹)	2.1	1.9	2.3	5.1	5.2	3.6
Min (ind.g ⁻¹)	0.1	0.2	0.0	0.6	0.2	0.6
Max (ind.g ⁻¹)	7.2	6.4	6.3	21.6	20.8	11.4
Average (ind.m ⁻²)	56 716	56 615	57 654	93 952	96 187	48 548
Min (ind.m ⁻²)	3 000	3 000	0	6 000	8 000	6 000
Max (ind.m ⁻²)	390 000	310 000	192 000	404 000	304 000	150 000
Average (ind.sample ⁻¹)	71	147	72	117	120	61
Min (ind.sample ⁻¹)	4	19	0	8	11	7
Max (ind.sample ⁻¹)	487	434	240	505	379	188
Number of observed species	10	14	12	18	10	18

Table 3. Complete list of taxa observed throughout the years 2020–2022 with total numbers of observed individuals per each taxon during the whole investigation period and dominance (%) recorded for individual sampling events.

Taxon	Trophic group	Numbers observed 2020–2022	Spring 2020 (%)	Autumn 2020 (%)	Spring 2021 (%)	Autumn 2021 (%)	Spring 2022 (%)	Autumn 2022 (%)
<i>Milnesium</i> sp.	Omni/carnivore	4		1.6	0.3			0.4
<i>Diphascon</i> cf. <i>bidropion</i> Ito, 1995	Microbivore	16		3.2		2.7		1.1
<i>Diphascon</i> cf. <i>higginsii</i> Binda, 1971	Microbivore	5			0.9			0.7
<i>Diphascon nobilei</i> agg.	Microbivore	2	4.9			0.6		
<i>Diphascon pingue</i> agg.	Microbivore	64	7.5	1.6	8.2	1.8	4.7	5.8
<i>Diphascon</i> aff. <i>speciosum</i> Mihelčič, 1971	Microbivore	29		0.8		6.2		2.9

Table 3. Continued.

Taxon	Trophic group	Numbers observed 2020–2022	Spring 2020 (%)	Autumn 2020 (%)	Spring 2021 (%)	Autumn 2021 (%)	Spring 2022 (%)	Autumn 2022 (%)
<i>Hypsibius sp.</i>	Herbivore	53	2.0	3.2	1.2	5.3	0.7	9.0
<i>Hypsibius cf. exemplaris</i> Gąsiorek, Stec, Morek & Michalczyk, 2018	Herbivore	2				0.6		
<i>Hypsibius aff. scabropygus</i> Cuénot, 1929	Herbivore	6	0.2			1.8		
<i>Adropion belgicae</i> Richters, 1911	Microbivore	18			4.5	0.9		
<i>Adropion scoticum</i> Murray, 1905	Microbivore	338	30.5	31.7	25.8	17.2	34.3	21.6
<i>Astatumen trinacriae</i> Arcidiacono, 1962	Microbivore	138	14.2	3.2	10.3	10.7	9.7	12.6
<i>Guidettion prorsirostre</i> Thulin, 1928	Microbivore	59	1.8	3.2	5.2	7.1	2.7	2.5
<i>Mesocrista revelata</i> Gąsiorek, Stec, Morek, Zawierucha, Kaczmarek, Lachowska-Cierlik & Michalczyk, 2016	Microbivore	349	42.8	10.3	27.6	20.5	33.0	28.8
<i>Fontourion recamieri</i> Richters, 1911	Microbivore	9		1.6	0.3	1.8		0.4
<i>Notahypsibius sp.</i>	Herbivore	2						0.4
<i>Ramazzottius sp.</i>	Herbivore	1		0.8				
<i>Dianea sp.</i>	Herbivore	25	0.9	0.8	14.2	3.3	1.0	3.6
<i>Isohypsibius sp.</i>	Herbivore	1						0.4
<i>Macrobiotus hufelandi</i> agg.	Omni/carnivore	119	5.1	38.1		6.5	12.0	3.2
<i>Mesobiotus sp.</i>	Omni/carnivore	65	0.2			13.1	2.0	5.4
<i>Paramacrobiotus richtersi</i> agg.	Omni/carnivore	4						1.4
<i>Dactylobiotus sp.</i>	Omni/carnivore	3			0.9			

Tardigrade communities in relation to tree dieback after bark beetle outbreak

Despite a general increase of tardigrade numbers after canopy dieback, the effect of canopy dieback had no significant effect on overall abundance (Table 4) due to the high variability of this trend observed among the studied plots (Fig. 4).

The average tardigrade abundance in the forest stands with live canopy was 3 ind/g dry soil (80 700 ind/m²) and gradually increased to 4 ind/g dry soil (92 500 ind/m²) in the first year and to 5 ind/g dry soil (56 000 ind/m²) in the second year after canopy dieback (Fig. 3).

In most of the infested plots, a peak of the abundance was observed one year after canopy dieback (P001, P003, P004, P005, P006) as evidenced by Fig. 4. Only at plot P002 the peak of the abundance was observed half a year later, i.e. 1.5 years after dieback. Peaks of the abundance were observed also in the plots with live canopy, although in variable years (i.e., A2021 for P008, P009, and S2022 for P007). Moreover, tardigrades in forests with live canopy achieved lower abundances during peaks, than in the plots with dead canopy layers (Fig. 4).

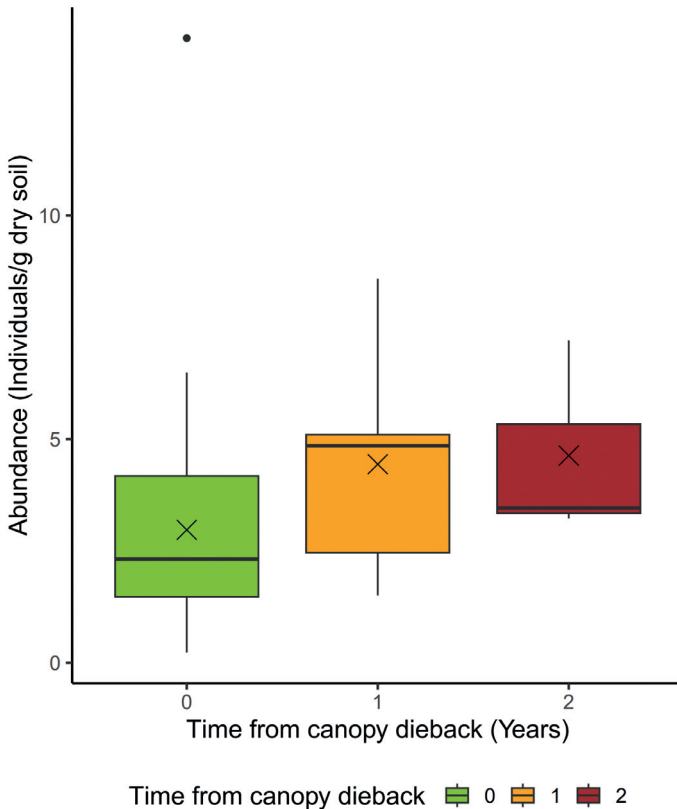


Fig. 3. Overall abundance in plots with live canopy (= 0), one (= 1) and two (= 2) years after canopy dieback. In boxplots the median is denoted by a bold horizontal line, the cross is the mean value, the interquartile range box represents the middle 50% of the data and the whiskers represent the full data range excluding outliers. Outlier is depicted by point.

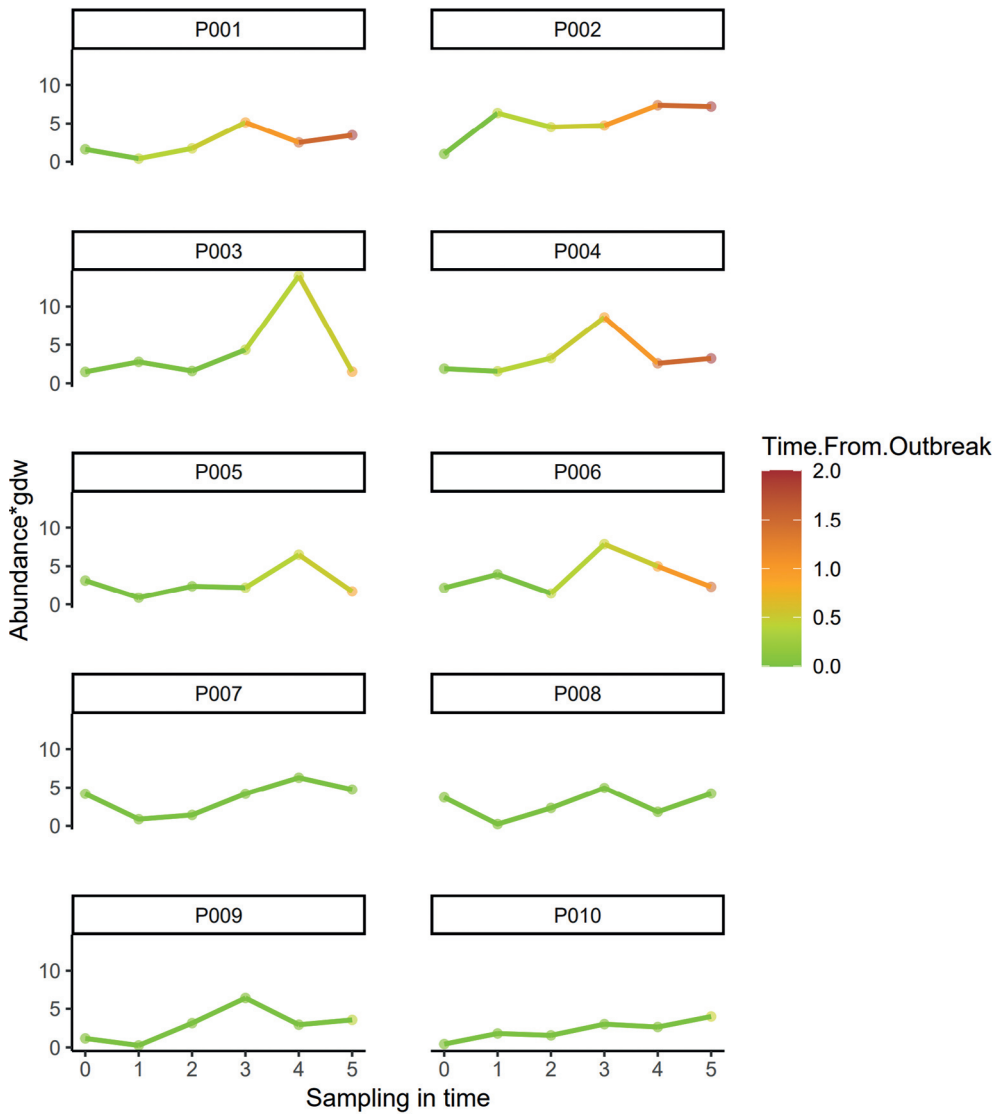


Fig. 4. Changes in tardigrade abundance (expressed as the number of individuals per 1g of sample dry weight) and canopy dieback (expressed by gradient colours; green = live canopy; brown = 2 years after canopy dieback) in the surveyed period on the individual investigated plots.

Table 4. Summary of the final linear mixed-effects (LME) model predicting the relationship between tardigrade abundance and the time from the canopy dieback, choice based on the lowest AIC. For the calculation of the probability of the t-value, the model was refitted by the method of Maximum Likelihood instead of Restricted Maximum Likelihood and probability calculated in the lmerTest package.

Linear mixed model fit by REML ['lmerMod']				
Formula: LogAbundance.per.gdw ~ TimeFromOutbreak + (1 Time)				
REML criterion at convergence: 240.1				
Random effects:				
Groups		Variance		Std.Dev.
Time		0.01832		0.1353
Residual		0.24454		0.4945
Number of obs: 160, groups: Time, 6				
Fixed effects:				
Factor	Estimate	Std.Error	t-value	p (lmerTest refitted by ML)
(Intercept)	0.24691	0.07407	3.333	0.0175*
TimeFromOutbreak	0.10043	0.07283	1.379	0.1709

Plot P010 was somehow exceptional since the abundance there was steadily increasing since the beginning till the end of the study, but it was hard to categorize the canopy dieback in plot P010 since half of the trees died and half of them were still alive at the end of the experiment.

Changes in Shannon diversity index in time from canopy dieback were statistically insignificant ($p = 0.437$). However, some of the taxa reacted to canopy dieback by changing their proportion in the community (Fig. 5). Specifically, *Mesocrista revelata*, *Diphascon* cf. *bidropion*, and *Diphascon pingue* agg. were represented by stable proportions during the canopy dieback. The dominance of *A. scoticum* and *Macrobotus hufelandi* agg. decreased, while proportions of *Astatumen trinacriae*, *Hypsibius* sp., and *Diphascon* aff. *speciosum* increased throughout the first two years after canopy dieback. *Guidettion prorsirostre*, *Mesobotus* sp., and *Dianeia* sp. slightly increased their proportions in the community shortly after the dieback, but two years after canopy dieback they were represented by similar proportions in the community as in plots with live canopy. For the other taxa enough observations are lacking to make conclusions on the relationship between their representation in the community and the canopy dieback.

As for the role of tardigrades in the food web, there were no significant changes in the proportions of individual trophic groups (Fig. 6). Microbivores formed a dominant group that on average accounted for 77% of the community. The average proportions of omnivores and herbivores were 15% and 8%, respectively. The biggest change was observed one year after

the canopy dieback when the mean proportion of microbivores slightly decreased to 62% at the expense of omnivores and herbivores, i.e. their mean proportion increased to 23% and 15%, respectively.

DISCUSSION

We observed no important effect of canopy dieback on the soil tardigrade community in the first two years after bark beetle infestation. Although, based on the graphs, there was a visible increase in tardigrade abundance connected to the start of the dieback and the changes in the species composition, however all these effects were statistically insignificant. Nonetheless, statistical insignificance may stem from the low number of samples evaluated after canopy dieback. Due to gradual infestation, plots were infested in variable time and some of them stayed alive till the end of the survey. By the end of 2022, we had 100 samples from plots

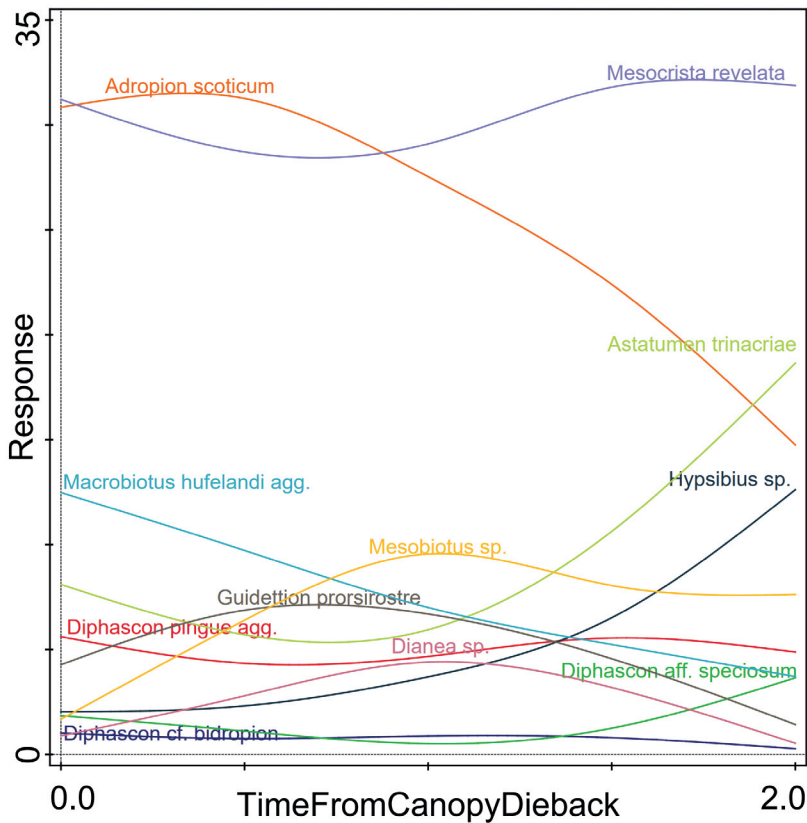


Fig. 5. Species response curves fitted by GAM. Changes in representation of each species in the community of tardigrades (in percentages) on the gradient of canopy dieback 0 = live canopy, 2 = 2 years after canopy dieback.

with live canopy, and only 20 samples measured 0.5 year after dieback, 18 samples 1 year after dieback, 12 samples 1.5 years after dieback, and only 9 samples 2 years after dieback. For more decisive results, longer measurements are needed to increase the number of samples connected with later stages of canopy dieback.

To our knowledge, there is no published study investigating the effect of canopy dieback on soil tardigrade communities. However, there are few studies considering clear-cut management. Nevertheless, their results seem contradictory. SOHLENIUS (1982) documented higher abundances first year after clear-cutting (compared to live undisturbed forest), while HUHTA (1976) observed the opposite pattern, i.e. lower abundances in the first year after clear-cutting. Still, clear-cutting is incomparably more intensive disturbance than bark beetle outbreak. Mechanical removal of trees includes soil cover and structure disturbance and pronounced changes in soil moisture and temperature, whereas most of these variables remain similar to the levels in the undisturbed forest after bark beetle outbreak (HAIS & KUČERA 2008, MA et al. 2010). Not all individuals were probably extracted using modified Baermann funnel technique. Still the abundances observed in the current study corresponded to common abundances reported for coniferous forests worldwide (e.g. HUHTA & KOSKENNIEMI 1975, HUHTA 1976, SOHLENIUS 1979, PERSSON et al. 1980, ITO 1999, ITO & ABE 2001, HARADA & ITO 2006).

One of the variables changing during bark beetle outbreak is nitrogen content and therefore, the most responsive group of organisms to these changes are microbivores (e.g. LOVETT & RUESINK 1995, ŠANTRŮČKOVÁ et al. 2010). In our study, most of the tardigrade taxa belonged to microbivores as is commonly observed in the coniferous forests (HARADA & ITO 2006), but we observed no significant increase in microbivorous tardigrades proportion

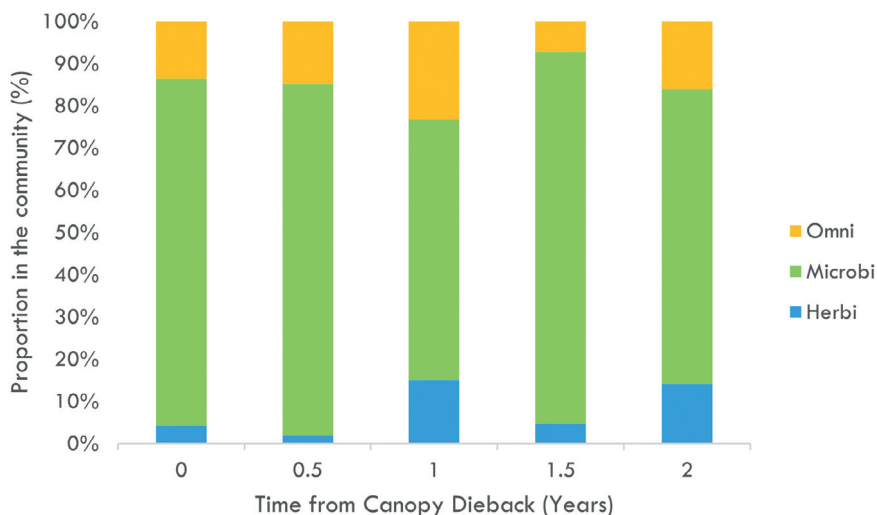


Fig. 6. Proportion of trophic groups in the tardigrade community in dependence on time from the canopy dieback.

after canopy dieback. In agreement with our results, the proportion of herbivores in soil tardigrade communities is around 5% of the observed individuals (HALLAS & YEATES 1972, HYVÖNEN & PERSSON 1996). Omnivores can have variable proportions. JÖNSSON (2003) even reported that the majority of tardigrades recorded in the coniferous forests in Sweden were omnivores. However, he observed the community inhabiting mosses, which usually differ to a wide extent from soil communities (e.g. BERTOLANI & REBECCHI 1996, GUIDETTI et al. 1999, GUIDETTI & BERTOLANI 2001, NELSON et al. 2020).

Sampling on closely positioned plots provided sufficient density and numbers of sample replications to cover tardigrade diversity and abundance in the area. On the other hand, our results relate to a small area, and the studied patches (20×20 m) that are only tens or hundreds of metres from each other may result in the reflection of factors influencing the whole studied areas no matter of canopy dieback at individual plots. For example, above the investigated area, there was a 500 m wide strip of forest that was cut down, and the nutrients that were possibly washed away from the strip might influence our plots positioned lower on the slope of the Smrčina hill.

Sufficient density of sampling is important to spot rare species with conservation significance. Some of the rarely observed taxa in our dataset might primarily inhabit other microhabitats than soil, for example, mosses or freshwater habitats (e.g. *H. cf. exemplaris*, *Dactylobiotus* sp.). However, at least one rare species probably represents a soil-dwelling animal with potential conservation significance. *Diphascion bidropion* has been described in Japanese mountain forests dominated by tsuga in 1995 (ITO 1995). Since then, it has not been recorded anywhere else. Due to the small number of soil-focused studies on tardigrades, *D. bidropion* may represent an overlooked, but common mountain forest species or it may also belong among rare species. In the second case, the natural zone Smrčina would then represent an important refugium for such species. Moreover, considering existing knowledge on tardigrade biogeography, there is a high probability that the *D. cf. bidropion* observed in Smrčina natural zone represents a new species closely related to Japanese *D. bidropion*.

In summary, the immediate changes in forest soils after bark beetle outbreak did not cause any visible effect on soil tardigrade communities. However, as the canopy collapse progresses, more observations on later stages of canopy dieback will be available and we can expect to observe more distinct changes in tardigrade community. We provide valuable information on soil tardigrade communities structure inhabiting mountain spruce forests and also, we are among rare studies on soil-inhabiting tardigrades, showing the community and trophic group structure in the European forests.

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