

Highly reduced mass loss rates and increased litter layer in radioactively contaminated areas

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Abstract The effects of radioactive contamination from Chernobyl on decomposition of plant material still remain unknown. We predicted that decomposition rate would be reduced in the most contaminated sites due to an absence or reduced densities of soil invertebrates. If microorganisms were the main agents responsible for decomposition, exclusion of large soil invertebrates should not affect decomposition. In September 2007 we deposited 572 bags with uncontaminated dry leaf litter from four species of trees in the leaf litter layer at 20 forest sites around Chernobyl that varied in background radiation by more than a factor 2,600. Approximately one quarter of these bags were made of a fine mesh that prevented access to litter by soil invertebrates. These bags were retrieved in June 2008, dried and weighed to estimate litter mass loss. Litter mass

loss was 40 % lower in the most contaminated sites relative to sites with a normal background radiation level for Ukraine. Similar reductions in litter mass loss were estimated for individual litter bags, litter bags at different sites, and differences between litter bags at pairs of neighboring sites differing in level of radioactive contamination. Litter mass loss was slightly greater in the presence of large soil invertebrates than in their absence. The thickness of the forest floor increased with the level of radiation and decreased with proportional loss of mass from all litter bags. These findings suggest that radioactive contamination has reduced the rate of litter mass loss, increased accumulation of litter, and affected growth conditions for plants.

Keywords Background radiation · Chernobyl · Decomposition · Invertebrates · Microorganisms

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Introduction

Climate has a crucial influence on litter decomposition rates, both directly and indirectly through effects on litter chemistry (e.g., Aerts 1997; Berg et al. 1993; Robinson 2002). Bacteria and fungi play key roles as decomposers of newly dead plant material (Staaf 1980; Howard and Howard 1974, 1980; Berg and Ekbohm 1991). Initial decomposition is followed by decomposition of resistant tissues like cellulose, lignins and cutins by fungi of various kinds (Staaf 1980; Howard and Howard 1974, 1980; Berg and Ekbohm 1991). For example, many studies have demonstrated the importance of lignin as a regulating factor in the decomposition of leaf litter (review in Osono 2007). Detritivores and microbivores follow suit and consume both detritus and its microflora (Staaf 1980; Howard and Howard 1974, 1980; Berg and Ekbohm 1991), and their interactions affect the

abundance and the diversity of species (review in Brown 1995). Decomposition of organic matter is a function of leaching, fragmentation, and mineralization, making C, N, P, and other nutrients available to plants (e.g., Attiwill and Adams 1993; Kalbitz et al. 2000). High rates of release of N and P during decomposition benefit growing plants, and thousands of plant species are completely dependent on the outcome of competition with microbes for acquisition of essential nutrients such as N and hence for growth and survival (van der Heijden et al. 2008). Furthermore, free-living microbes strongly regulate plant productivity through mineralization of organic matter (van der Heijden et al. 2008). Soil invertebrates play a significant role in litter decomposition (e.g., Cornelissen et al. 1999; Pouyat et al. 1994; Gonzalez and Seastedt 2001). Therefore, when the community of soil microbes and invertebrates is seriously perturbed, as in the case of a major nuclear accident, such perturbation can have dramatic indirect effects on performance of plants and hence herbivores, but also on the mineralization of organic matter.

Although decomposition and mineralization of organic matter are of utmost importance for soils, there are only rudimentary analyses of the effects of radiation on soil communities at Chernobyl, Fukushima or other sites with elevated background radiation (Møller and Mousseau 2006). Pigeon and Odum (1970) experimentally irradiated a forest at Luquillo in Puerto Rico with gamma radiation and monitored the effects on litter decomposition. This study of acute high dose irradiation is of general interest although there was no replication of irradiated and control sites, exposures were limited to 3 months, the radiation was only from a single external source, and there was no ability to control for the many potentially confounding effects of radiation on other components of the study system. Hence it is difficult to make any generalization from this study. Rafferty et al. (1997) showed that fungus-mediated translocation of ^{137}Cs to fresh litter may explain the persistence of Chernobyl radiocaesium in the upper layers of forest soils. In contrast, a laboratory study of decomposition of irradiated wheat straw showed only weak effects of ionizing radiation (Niedree et al. 2012), perhaps suggesting that the consequences of short-term radiation in the lab differ from chronic levels of radiation for numerous generations of soil microorganisms and invertebrates under field conditions. Most soil and litter invertebrates were exposed to high levels of radiation for long periods after the Chernobyl disaster due to most radionuclides being concentrated in the topmost 10 cm of the soil. Krivolutski et al. (1999) showed that within months of the accident invertebrates at a distance up to 7 km from the nuclear power plant were reduced by up to a factor of 30. This reduction arose from extensive reproductive failure and heavy mortality of eggs and juvenile stages. The ratio of adult to juvenile stages

among soil invertebrates was restored in most areas after 2.5 years, although biodiversity still remained severely reduced due to the loss of many radio-sensitive species (Victorov 1993; Krivolutski and Pokarzhevsky 1992). For example, the abundance and diversity of soil invertebrates were severely reduced by up to a factor 30 at distances exceeding 3 km from the reactor just after the accident (Krivolutski and Pokarzhevsky 1992), and abundances were still depressed 20 years after the accident (Maksimova 2005). Effects on soil invertebrates in agricultural areas were weaker than in forests although radiation also affected abundance, diversity and reproduction of soil invertebrates in these habitats (Krivolutski et al. 1999). Therefore, it is not surprising that bird species with a main diet of soil invertebrates had highly reduced abundance in the most contaminated areas either due to contamination from soil invertebrates or due to reductions in the abundance of food (Møller and Mousseau 2007). Evidence suggests that the abundance of saprophytic bacteria in contaminated soils is reduced (Zymenko et al. 1995; Romanovskaya et al. 1998) although analyses of bacterial biofilms on building surfaces at Chernobyl and elsewhere found no reduction in abundance or biodiversity (Ragon et al. 2011). However, such surfaces are peculiar because of the general effects of ultra-violet radiation on biodiversity (Ragon et al. 2011).

The objectives of this study were to quantify litter mass loss from areas varying in level of contamination with radionuclides in the vicinity of Chernobyl. First, we placed litter bags with uncontaminated leaf litter in the litter layer in forest habitats varying in degree of contamination and compared the effects of radiation on leaf litter loss to variation in radiation within sites. Furthermore, we deposited 155 leaf litter bags with fine nylon mesh to prevent large invertebrates from gaining access to leaves while 400 bags in the same sites with coarse nylon mesh created the contrast between decomposition by the combination of invertebrates and microorganisms or by microorganisms alone. Second, we tested the extent to which differences in background radiation levels between a high- and a low-radiation site predicted differences in mass loss from litter bags between such pairs of sites. Such pairs of sites are likely to be very similar in many respects because they tend to share microclimate, soil type, soil pH, soil moisture, vegetation and many other characteristics. Third, we related forest floor thickness to radiation level, litter mass loss from the litter bag experiments and stand composition. We predicted that the forest floor would be thicker in sites with higher level of background radiation, in sites with reduced proportional mass loss, and in the presence of pine stands surrounding the area where we placed the litter bags because conifer needles can sometimes require a long time to decompose (Prescott et al. 2000; Albers et al. 2004).

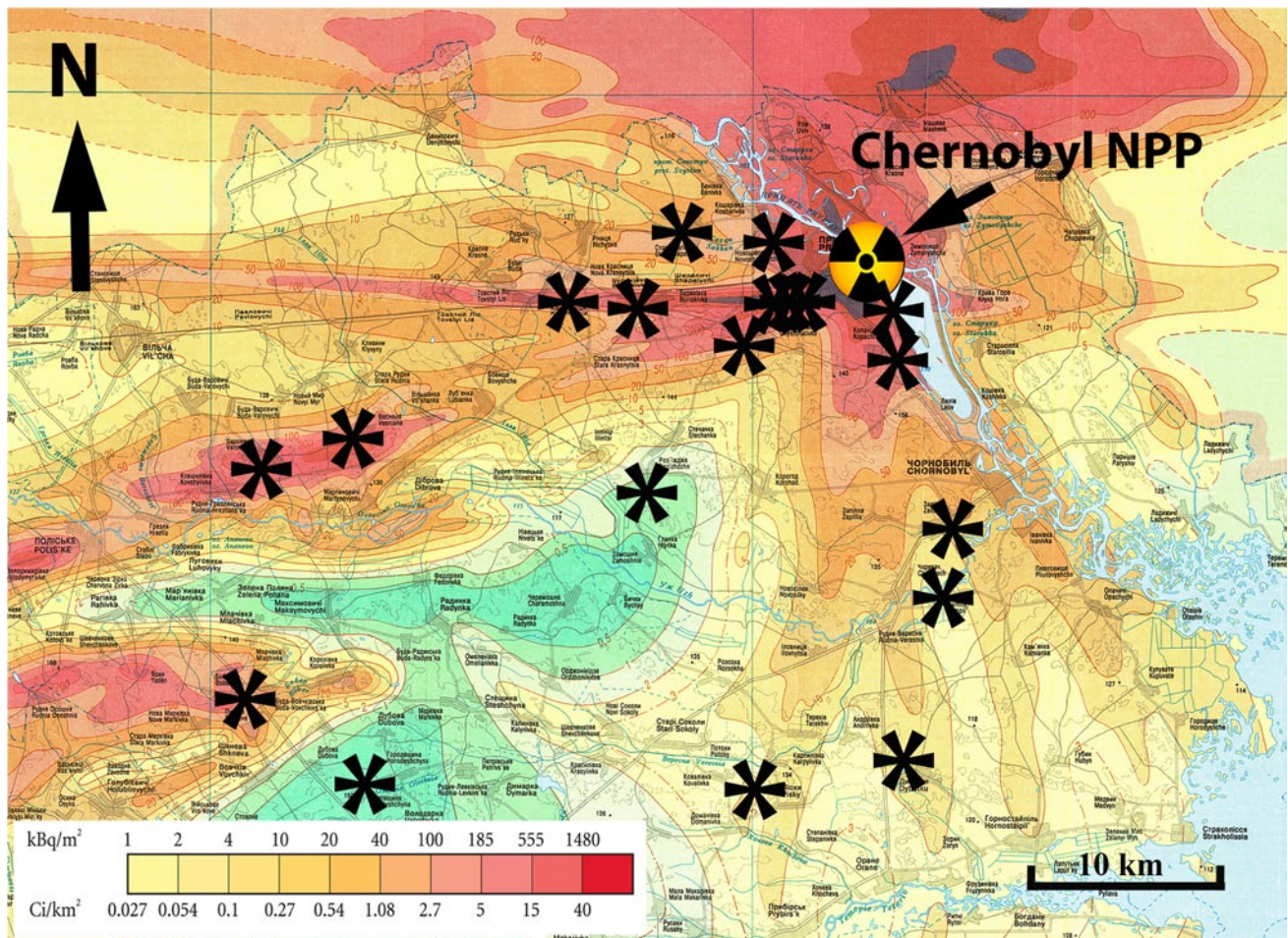


Fig. 1 Sites used for decomposition experiments in relation to background radiation level. Adapted from Shestopalov (1996)

Materials and methods

Study sites

We (wearing radiation protection suits in the most contaminated areas) deposited litter bags during September 2007 within the Chernobyl exclusion zone or in areas adjacent on the southern and western borders. Sites were selected to ensure a wide range of radiation levels (Fig. 1). Locations of the 20 study sites are shown in Fig. 1. The study sites were forests or successional stages of farmland that are presently reverting to forests and are located in dry soil with little variation in altitude or climate.

Litter mass loss experiments

We were interested in the early stages of decomposition because large amounts of accumulated litters suggest an effect of radiation on decomposition. In addition such early effects can be linked directly to the abundance

of vertebrates that consume soil invertebrates. We collected freshly abscised leaves of oak *Quercus robur*, maple *Acer platanoides*, or birch *Betula pendula* and needles of Scots pine *Pinus sylvestris* on the ground at a number of uncontaminated sites near Kiev, Ukraine during September 2007. Litter samples for each species were subsequently mixed to avoid any effects of origin of samples before deposition in the litter layer in the forests. Background radiation levels at the sites from where the leaves were collected ranged from 0.05 to 0.12 $\mu\text{Sv/h}$, and were hence within the range of natural levels of background radiation in Ukraine. Leaves were air dried at room temperature for about 2 weeks followed by additional drying in an oven at 60 °C for 24 h immediately before being weighed on a calibrated electronic balance to the nearest 0.01 g. Litter bags were then taken to the field sites and left in the soil for 9 months. This duration of exposure was considered to mimic the situation of leaf loss by deciduous trees in September–October in Chernobyl, and the almost complete disappearance of such leaves by mid-summer

the following year, unless radiation reduces or prevents decomposition.

We made nylon mesh bags sized ca. 15×20 cm with mesh size being 0.2 cm for depositing leaf litter in the field. This mesh size will exclude some large-sized macro-decomposer groups, and thus this constitutes a potential limitation of the study. Bags were filled with ca. 18 g leaf litter, range 10–31 g, before being closed with glue and placed in the leaf litter in a forest. We filled litter bags with litter in a cottage in the field. Since the mass of litter remaining at the end of the experiment was the response variable and the mass of litter at the beginning of the experiment was one of several predictor variables, the potential impact of differences in the amount of litter was controlled statistically in the analyses. Since all bags were deposited randomly, there was no reason to expect any systematic bias in our findings. Each bag was numbered with a unique identification number for later identification. We took great care to deposit all bags in a container before transport to the field, and likewise we took care not to lose any material from the bags during deposition. Although pine needles may be expected to more readily be lost from bags, this was not the case because needles were paired and hence got stuck in the mesh. Thus, needles were more difficult to remove from the bags than leaf litter at the end of the experiment. Bags were arranged in random order on blue plastic strings for ease of later detection, because wild boar *Sus scrofa* or other large animals may disturb single bags and such bags may hence be difficult or impossible to retrieve. In addition the coordinates were recorded with a Global Positioning System (GPS) and a blue plastic band tied to the nearest tree. Slightly more than a quarter of all bag contents was placed in a fine nylon mesh bag with the mesh being <0.5 mm to prevent invertebrates from gaining access to the leaf litter (e.g., Lousier and Parkinson 1976). The number of bags for each species reflected the availability of leaves of the different species at the uncontaminated sites near Kiev where the leaves were originally collected.

At each site we attempted to find the most and the least contaminated locations at a maximum distance of 100 m ensuring that we had at least two high-radiation and two low-radiation locations within a site. We removed the vegetation, deposited the bags in the litter layer of the forest and covered the bags with vegetation so that litter bags were less visible and thus less likely to be removed by humans or animals. All bags were located in the shade under trees. When depositing litter bags we simultaneously recorded the pH using a pH meter (Sharp PH51) and soil moisture using a Delta-T Thetaprobe and HH2 moisture data logging system by inserting the instruments into the leaf litter layer three to five times. The mean estimate for a location was used in the subsequent analyses. The 572 litter bags were located during June 2008 after having been left in forests

for 9 months. We only retrieved 560 bags, because 12 were lost due to activity of a tractor and a wild boar *S. scrofa*. An additional five bags were partly damaged resulting in a final sample of 555 bags. After collection of the bags in June 2008 leaf material was air dried at 60 °C for 48 h in a Fisher Isotemp oven and stored in sealed plastic containers containing Drierite silica gel prior to weighing on a Sartorius balance to the nearest 0.01 g. The number of different kinds of litter bags is reported in Electronic Supplementary Material Table S1, while Table S2 reports mean pH and soil moisture for low- and high-radiation locations within the 20 study sites (dividing locations into low- and high-radiation locations at the median).

Measuring thickness of the forest floor

During 28–31 May 2013 we returned to the exact same locations where the litter bags were deposited during September 2007 by using the GPS coordinates. At each of these locations we made four measurements of the thickness of the forest floor with a ruler to the nearest millimeter within a distance of 1 m. At each site we also recorded the presence or the absence of stands of pines in the exact spot where measurements were made because pine needles at least sometimes take a particularly long time to decompose, and because pines are particularly susceptible to the effects of background radiation (Arkhipov et al. 1994; Mousseau et al. 2013).

Measuring background radiation levels

We measured radiation levels in the field at ground level at each location where a litter bag was deposited using a hand-held dosimeter (Inspector; SE International, Summertown, TN). We measured levels two to three times at each of these locations and averaged the measurements for each location. These data were correlated with data from governmental measurements at ground level published by Shestopalov (1996), estimated as the mid-point of the ranges in the published maps. These analyses showed a high degree of consistency between the two methods (Møller and Mousseau 2007). Radiation levels vary by several orders of magnitude at a scale of 1 km due to heterogeneity in deposition of radioactive material since the Chernobyl accident (Fig. 1; Shestopalov 1996).

Statistical analyses

Initial leaf litter mass, final leaf litter mass, and radiation level were $\log_{10}$ transformed to normalize the data. We developed a full statistical model for all litter bags to assess the relationship between final leaf litter mass (response variable) and initial leaf litter mass, presence or absence of

Table 1 Summary statistics for soil and decomposition variables for the study of decomposition at Chernobyl

Variable	Mean	SE	CV	Range
Radiation ($\mu\text{Sv/h}$)	20.26	2.26	262.52	0.09–240.25
pH	5.24	0.05	23.87	3.1–7.8
Moisture (%)	10.16	0.17	39.38	3.1–21.0
Initial leaf litter mass (g)	17.81	0.15	19.51	10.00–31.20
Final leaf litter mass (g)	4.86	0.10	47.47	0.26–13.95
Proportional loss relative to initial mass	0.273	0.005	21.07	0.02–0.88
Thickness of forest floor (cm)	6.81	0.43	53.53	2.0–16.0

Sample size was 555 leaf litter bags. Proportional loss relative to initial mass represents the proportion of the mass of the litter that is lost from the initial mass of the litter

CV Coefficient of variation

a fine mesh bag, tree species litter, pH, soil moisture and radiation nested within site (predictors), as implemented in the statistical software JMP (SAS 2012). We nested the radiation variable within the categorical variable Sites because we were mainly interested in quantifying the effect of radiation on decomposition at each of the sites. Contrasts within sites were important because differences in decomposition related to radiation among sites could be due to confounding variables rather than radiation. This model included all the predictors that we had a priori biological reasons to include. The objective of this global model was to test whether mass loss was related to background radiation within study sites.

Second, we also tested this objective in a more conservative statistical model with the mean amount of remaining

leaf litter for each of the 20 study sites being the response variable and mean background radiation level being the predictor variable. This model was used to test whether mean mass loss was related to mean background radiation level across the 20 different sites.

Third, an even more conservative statistical model was used to test the same hypothesis based on pairs of spatially neighboring study sites ranked with respect to radiation. We related the difference between two neighboring sites in mean loss of leaf litter to the difference in mean background radiation between these two neighboring sites. The justification for this analysis is that it controls for effects of other potentially confounding variables because neighboring sites differing in level of background radiation generally are similar in terms of microclimate, soil type, soil pH, soil moisture, vegetation and other variables of potential significance.

For the thickness of the forest floor we developed a model with thickness of the forest floor as the response variable and background radiation, proportional mass loss from the litter and presence or absence of pine trees as predictor variables. We predicted a positive effect of background radiation on the thickness of the forest floor if radiation had a negative impact on decomposers. In contrast, we predicted a negative effect of mass loss from litter bags on thickness of the forest floor because greater mass loss should imply less litter. Finally, we predicted a positive effect of presence of pine stands in the areas surrounding the area where we measured the thickness of the forest floor because pine litter at least sometimes takes longer to decompose than litter from deciduous trees.

Table 2 Final leaf litter mass in relation to radiation level (nested within sites), site, initial leaf litter mass, tree species and presence or absence of a fine mesh bag

Variable	Sum of squares	df	F	P	Slope (SE)
Radiation(Site)	0.235	17	2.13	0.0054	
Site	0.504	16	4.85	<0.0001	
Initial leaf litter mass	1.062	1	163.59	<0.0001	0.784 (0.061)
Tree species	0.415	3	21.30	<0.0001	
Least square means SE					
	Pine	3.144	0.044		
	Oak	3.135	0.044		
	Maple	3.119	0.044		
	Birch	3.079	0.044		
Fine mesh bag	0.194	1	29.87	<0.0001	
Least square means SE					
	Fine mesh bag	3.147	0.044		
	No fine mesh bag	3.093	0.043		
Error	3.342	515			

The full model had the statistics $F = 18.99$, $df = 39, 515$, $r^2 = 0.59$, $P < 0.0001$

Results

Mass loss from litter

Summary statistics for the different soil and litter mass loss variables and thickness of the forest floor are reported in Table 1. Radiation was the variable with the largest coefficient of variation due to the choice of sites varying greatly in background radiation level. pH was on average 5.2 with most values being acidic. The mean proportional loss relative to initial leaf mass was 27 %.

A full model based on all litter bags explained 59 % of the variance (Table 2). The model fitted the data as shown by the non-significant lack of fit ($F = 0.99$, $df = 505$, 9 , $P = 0.57$). There was a significant effect of radiation nested within sites, with an additional effect of site (Table 2). Unsurprisingly, initial leaf litter mass also accounted for a significant effect with larger amounts being present at the end of the experiment when a larger amount was present at the start of the experiment. Tree species differed in rate of litter mass loss, ranked from slowest to quickest as pine, oak, maple and birch (Table 2). A Tukey post hoc test revealed that birch differed significantly ($P < 0.05$) from the three other species, while there were no other significant differences. The effect of fine mesh on litter mass loss was significant implying that access to the leaves by large invertebrates increased the rate of litter mass loss (Table 2). Two separate analyses for the litter bags with and without fine mesh net similar to the statistical model in Table 2 produced a significant effect of radiation in the absence of fine mesh ($F = 2.53$, $df = 1$, 365 , $P = 0.0008$), but produced no significant effect of radiation in its presence ($F = 1.13$, $df = 1$, 118 , $P = 0.34$). There was no additional significant effect of pH ($F = 3.58$, $df = 1$, 514 , $P = 0.06$) or soil moisture ($F = 0.46$, $df = 1$, 514 , $P = 0.50$). A model that included a non-linear effect by inclusion of a quadratic radiation term did not provide a significant effect for that variable ($F = 0.00$, $df = 1$, 514 , $P = 0.97$). Therefore, there was no evidence of a radiation threshold or a non-linear effect. There was no statistically significant interaction between radiation and tree species when this interaction was entered into the model in Table 2 ($F = 0.62$, $df = 3$, 512 , $P = 0.61$). This implies that radiation did not have a different effect on mass loss in the four different tree species. Likewise, there was no statistically significant interaction between radiation and presence or absence of fine mesh when this interaction was entered into the model in Table 2 ($F = 0.04$, $df = 1$, 512 , $P = 0.85$). This implies that the effect of radiation on mass loss was not significantly different in the absence or the presence of fine mesh and hence soil invertebrates. Thus, the effects of radiation on mass loss were independent of tree species and presence or absence of fine mesh.

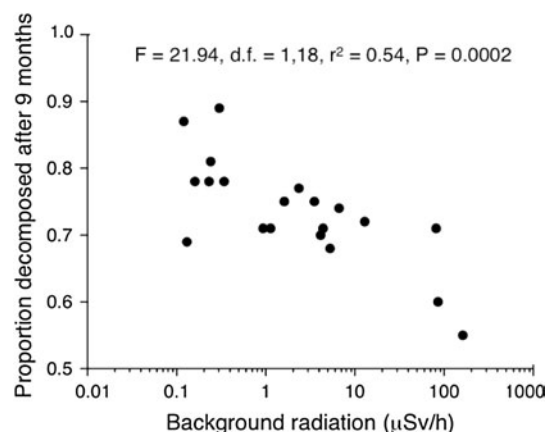


Fig. 2 Decomposition estimated as the proportion of initial leaf litter mass decomposed in relation to measured background radiation level ($\mu\text{Sv/h}$) for the study sites. Statistics for the model are reported

A more conservative statistical model that related the mean mass of leaf litter remaining at each study site to the mean level of radiation accounted for 55 % of the variance [Fig. 2; $F = 21.94$, $df = 1$, 18 , $r^2 = 0.54$, $P = 0.0002$, slope (SE) = -0.070 (0.015)]. The difference in litter mass loss between the site with the highest and the lowest radiation level estimated from the predicted values according to the regression line was 40 %.

Mean reduction in leaf litter mass was 0.249 (SE = 0.010) for the ten high-radiation sites and 0.276 (0.012) for the ten low-radiation sites. This difference of 11 % was statistically significant (paired t -test, $t = 2.73$, $df = 9$, $P = 0.023$). In fact the difference in reduction in leaf litter mass between neighboring sites was negatively related to the difference in radiation level between neighboring sites [$F = 11.55$, $df = 1$, 8 , $r^2 = 0.59$, $P = 0.0094$, slope (SE) = -0.021 (0.006)]. Therefore, the conclusion that radiation reduced the rate of litter mass loss remained across all three statistical tests.

Thickness of forest floor

A model of thickness of the forest floor in relation to background radiation level, proportional mass loss from litter bags, and presence or absence of pine stands fitted the data. These three factors contributed to explaining variation in thickness of the litter layer (Table 3). The thickness of the forest floor increased with increasing level of background radiation (Fig. 3a). In addition, there was a significant effect of proportional loss of mass from litter bags (Fig. 3b), implying that the forest floor was thicker in sites where proportional loss was reduced. Finally, there was a tendency for an increase in thickness of the forest floor in sites with stands of pines (Table 3).

Table 3 Model of the relationship between thickness of the forest floor and background radiation, proportional loss of litter mass, and presence of absence of stands of pine trees

	Sum of squares	<i>F</i>	<i>df</i>	<i>P</i>	Estimate	SE
Intercept		78.323	1	<0.0001	2.483	0.281
Background radiation	1.080	83.684	1	<0.0001	0.127	0.014
Proportional loss	0.521	40.359	1	<0.0001	−3.352	0.543
Presence of pine trees	0.051	3.954	1	0.051	0.027	0.014
Error	0.890		69			

The overall model had the statistics $F = 66.45$, $df = 3, 69$, $r^2 = 0.74$, $P < 0.0001$

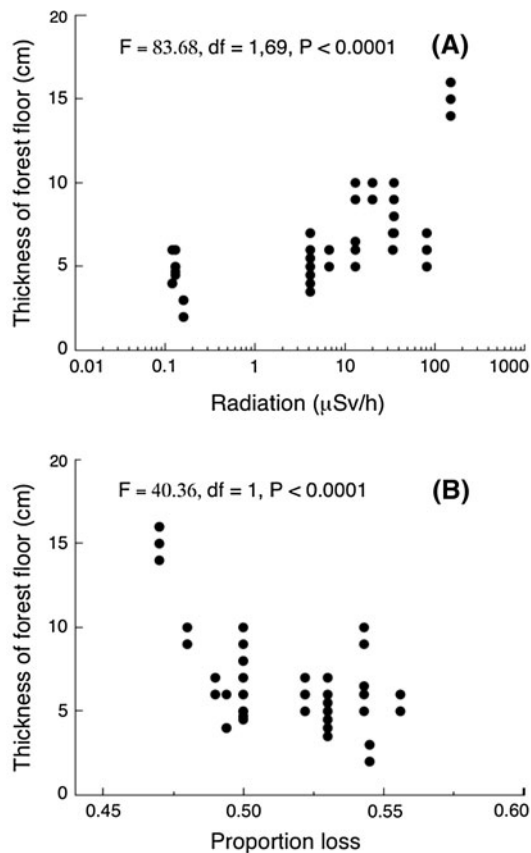


Fig. 3 Thickness of the forest floor (cm) within study sites in relation to **a** background radiation level at the study sites ($\mu\text{Sv/h}$) and **b** proportional loss of litter mass at the study sites. Statistics for the partial effects for the model are reported

Discussion

The main findings of this study were that decomposition as reflected by mass loss of leaf litter decreased with increasing level of background radiation in forest sites around Chernobyl resulting in a reduction by 40 % across a radiation gradient differing in radiation level by more than a factor 2,600. Potentially confounding variables such as site effects, effects of invertebrates, pH or soil moisture did

not change the conclusions concerning effects of radiation on litter mass loss. We reached similar conclusions when analyzing individual litter bags within sites, mean values of litter mass loss for sites, or differences in litter mass loss between pairs of neighboring sites differing in level of radiation. This shows that our findings were robust and independent of the spatial scale of analysis. Finally, mass loss from litter bags had consequences for litter mass loss and accumulation of organic matter in the field because the forest floor was thicker in sites with higher levels of background radiation, lower proportional mass loss from litterbags and sites with stands of pine trees.

We estimated the impact of radiation on litter mass loss by comparing the rate at the highest radiation level relative to the rate at the lowest background radiation level. The decrease in rate of litter mass loss was by 40 % in the most contaminated sites compared to the cleanest sites. While each of our bags with leaf litter only had leaves from one tree species, patterns of leaf litter mass loss, changes in nutrient concentration, and decomposer abundance and activity depend on whether leaves are decaying in mixtures or on their own (Gartner and Cardon 2004). Therefore, future studies based on mixed leaf litter may provide results that are more similar to the situation in natural forests.

A quarter of our litter bags were enclosed in fine nylon mesh to prevent access by large soil invertebrates. While there is clear evidence of the soil fauna being perturbed by radioactive contamination from Chernobyl (Krivolutski et al. 1999; Victorov 1993; Krivolutski and Pokarzhevsky 1992; Maksimova 2005), the information on soil microbes is rudimentary at best. Ragon et al. (2011) showed similar abundance and diversity of bacteria in biofilms on building surfaces in Chernobyl and elsewhere in Europe, although the rate of mutation accumulation was increased in the most contaminated areas in Chernobyl. Zymenko et al. (1995) and Romanovskaya et al. (1998) reported reduced abundance of saprophytic bacteria in more contaminated soils. We assumed that both microorganisms and soil invertebrates have access to litter bags with coarse mesh, while only microorganisms have access to litter bags with fine mesh. We found a significant difference of <1 % in litter

mass loss between bags with and without fine mesh. Since we did not find a significant interaction between presence or absence of fine mesh and background radiation, we conclude that a given level of radiation had an equally negative impact on litter bags with and without mesh.

We found differences in the rate of litter mass loss among tree species with greater loss in birch than in the three other species. Such differences between conifers and deciduous trees are well described in the literature (e.g., Berg and Ekbohm 1991; Gillon et al. 1994; Taylor and Parkinson 1988). These differences in litter mass loss among tree species are as expected. Surprisingly, we did not find a significant interaction between tree species and background radiation on level of mass loss from litter. This finding suggests that all species were equally impacted by background radiation, and it further suggests that it is the decomposers rather than the litter that are affected by the effects of background radiation. An experiment that consisted of litter bags with both clean and contaminated litter would allow us to determine whether contamination effects on litter quality affect rates of decomposition.

While mass loss from litter bags is only one of several components of decomposition, the ultimate test of an effect of background radiation on decomposition is through the effects on the thickness of the forest floor. We made such a test in 2013. The experiment on mass loss from litter bags was made in 2007–2008, so there is a temporal delay between the two parts of this study. We see no obvious reason why this should be a cause of consistent bias, and it is our clear impression based on extensive field work in 1991, 1995 and 2000–2013 that the thickness of the forest floor is consistently positively related to background radiation every year. Here we have shown that the thickness of the forest floor increased with the level of background radiation.

We have conducted research in Chernobyl since 1991 and have noticed a significant accumulation of litter over time. This accumulation of litter is demonstrated here to be significantly positively related to background radiation, negatively related to the proportional loss of litter mass from the litter bags, and to be greater in the presence of pine stands. Accumulation of litter may have consequences for the risk of fire because accumulation of dead plant matter for 27 years implies an accumulation of fuel and hence an elevated risk of fire followed by an increased risk of subsequent redistribution of radionuclides. Obviously, factors other than litter accumulation will impact the risk of fire. These include the probability of fire ignition, which will have been affected by increased summer temperatures in recent decades, reduced levels of precipitation, the large spatial scale of the areas with accumulation of litter, the reduced levels of defense against insect pests and the resultant rate of tree mortality (A. P. Møller et al., unpublished

data). Several studies have suggested non-negligible risks of a major Chernobyl fire (e.g., Kashparov et al. 2000; Yoschenko et al. 2006a, b), and our study provides important information on factors that relate to this risk. The accumulation of litter over time may also have consequences for the mineralization of organic matter in the forest floor and hence primary productivity, suggesting that growth rates of trees and other plants may be suppressed in the presence of poor levels of decomposition caused by elevated levels of background radiation and could in part explain reduced growth rates of pine trees in radioactive regions of Chernobyl (Mousseau et al. 2013).

In conclusion, we have shown severely depressed levels of litter mass loss in the most contaminated forest areas around Chernobyl, with reductions of 40 % relative to background levels recorded in uncontaminated, control areas. These findings suggesting a linear dose–response of decomposition in relation to background radiation were independent of potentially confounding variables such as pH, soil moisture, study site and method of analysis. These results have a number of implications for management of contaminated areas, risk of fire and redistribution of radionuclides in forest ecosystems around Chernobyl.

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References

- Aerts R (1997) Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* 79:439–449
- Albers D, Migge S, Schaefer M, Scheu S (2004) Decomposition of beech leaves (*Fagus sylvatica*) and spruce needles (*Picea abies*) in pure and mixed stands of beech and spruce. *Soil Biol Biochem* 36:155–164
- Arkhipov NP, Kuchma ND, Askbrant S, Pasternak PS, Musica VV (1994) Acute and long-term effects of irradiation on pine (*Pinus silvestris*) stands post-Chernobyl. *Sci Total Environ* 157:383–386
- Attwill PM, Adams MA (1993) Nutrient cycling in forests. *New Phytol* 124:561–582
- Berg B, Ekbohm G (1991) Litter mass-loss rates and decomposition patterns in some needle and leaf litter types: long-term decomposition in a Scots pine forest. *Can J Bot* 69:1449–1456
- Berg B, Berg MP, Bottner P, Box E, Breymeyer A, Calvo de Anta R, Coueaux M, Escudero A, Gallardo A, Kratz W, Madeira M, Mälkönen E, McClaugherty C, Meentemeyer V, Muñoz F, Piussi P, Remacle J, Virzo de Santo A (1993) Litter mass loss rates in pine forests of Europe and Eastern United States: some relationships with climate and litter quality. *Biogeochemistry* 20:127–159
- Brown GG (1995) How do earthworms affect microfloral and faunal community diversity? *Plant Soil* 170:209–231

- Cornelissen JHC, Perez-Harguindeguy N, Diaz S, Grime JP, Marzano B, Cabido M, Vendramini F, Cerabolini B (1999) Leaf structure and defence control litter decomposition rate across species and life forms in regional floras on two continents. *New Phytol* 143:191–200
- Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. *Oikos* 104:230–246
- Gillon D, Joffre R, Ibrahima A (1994) Initial litter properties and decay rate: a microcosm experiment on Mediterranean species. *Can J Bot* 72:946–954
- Gonzalez G, Seastedt TR (2001) Soil fauna and plant litter decomposition in tropical and subtropical forests. *Ecology* 82:955–964
- Howard PJA, Howard DM (1974) Microbial decomposition of three and shrub leaf litter. *Oikos* 25:341–352
- Howard PJA, Howard DM (1980) Effect of species, source of litter, type of soil, and climate on litter decomposition: microbial decomposition of three and shrub leaf litter. *Oikos* 34:115–124
- JMP (2012) JMP version 10.0. SAS Institute, Cary, NC
- Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Sci* 165:277–304
- Kashparov VA, Lundina SM, Kadygriba AM, Protsaka VP, Levchuka SE, Yoschenkoa VI, Kashpurb VA, Talerko NM (2000) Forest fires in the territory contaminated as a result of the Chernobyl accident: radioactive aerosol resuspension and exposure of firefighters. *J Environ Radioact* 51:281–298
- Krivolutski DA (2000) Problems of sustainable development and ecological indication in radioactively contaminated areas. *Russ J Ecol* 31:233–237
- Krivolutski DA, Pokarzhevsky AD (1992) Effect of radioactive fallout on soil animal populations in the 30 km zone of the Chernobyl NPP. *Sci Total Environ* 112:69–77
- Krivolutski D, Martushov V, Ryabtsev I (1999) Influence of radioactive contamination on fauna in the area of the Chernobyl NPP during first years after the accident (1986–1988). *Bioindicators of radioactive contamination*. Nauka, Moscow, pp 106–122
- Lousier JD, Parkinson D (1976) Litter decomposition in a cool temperate deciduous forest. *Can J Bot* 54:419–436
- Maksimova S (2005) Radiation effects on the populations of soil invertebrates. In: Brechignac F, Desmet G (eds) *Equidosimetry*. Springer, Berlin, pp 155–161
- Møller AP, Mousseau TA (2006) Biological consequences of Chernobyl: 20 years after the disaster. *Trends Ecol Evol* 21:200–207
- Møller AP, Mousseau TA (2007) Species richness and abundance of birds in relation to radiation at Chernobyl. *Biol Lett* 3:483–486
- Mousseau TA, Welch SM, Chizhevsky I, Bondarenko O, Milinevsky G, Tedeschi DJ, Bonisoli-Alquati A, Møller AP (2013) Tree rings reveal extent of exposure to ionizing radiation in Scots pine *Pinus sylvestris*. *Trees* 27:1443–1453
- Niedree B, Vereecken H, Burauel P (2012) Effects of low-level radioactive soil contamination and sterilization on the degradation of radiolabeled wheat straw. *J Environ Radioact* 109:29–35
- Osono T (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecol Res* 22:955–974
- Pigeon RF, Odum HT (eds) (1970) *A tropical rain forest; a study of irradiation and ecology at El Verde, Puerto Rico*. United States Atomic Energy Commission, National Technical Information Service
- Pouyat RV, Parmelee RW, Carreiro MM (1994) Environmental effects of forest soil invertebrates and fungal densities in oak stands along an urban-rural land-use gradient. *Pedobiologia* 38:385–399
- Prescott CE, Zabek LM, Kabzems R (2000) Decomposition of broadleaf and needle litter in forests in British Columbia: influence of litter type, forest type, and litter mixtures. *Can J For Res* 30:1742–1750
- Rafferty B, Dawson D, Kliashtorin A (1997) Decomposition in two pine forests: the mobilisation of ^{137}Cs and K from forest litter. *Soil Biol Biochem* 29:1673–1681
- Ragon M, Restoux G, Moreira D, Møller AP, López-García P (2011) Sunlight-exposed biofilm microbial communities are naturally resistant to Chernobyl ionizing-radiation levels. *PLoS One* 6(7):e21764
- Robinson CH (2002) Controls on decomposition and soil nitrogen availability at high latitudes. *Plant Soil* 242:65–81
- Romanovskaya VA, Sokolov IG, Rokitko PV, Chernaya NA (1998) Ecological consequences of radioactive contamination for soil bacteria in the 10 km Chernobyl zone. *Microbiol* 67:274–280
- Shestopalov VM (1996) *Atlas of Chernobyl exclusion zone*. Ukrainian Academy of Science, Kiev
- Staaf H (1980) Influence of chemical composition, addition of raspberry leaves, and nitrogen supply on decomposition rate and dynamics of nitrogen and phosphorus in beech leaf litter. *Oikos* 35:55–62
- Taylor BR, Parkinson D (1988) Aspen and pine leaf litter decomposition in laboratory microcosms: interactions of temperature and moisture level. *Can J Bot* 66:1966–1973
- van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296–310
- Victorov AG (1993) Radio-sensitivity and radio-pathology of earthworms and their use as bio-indication of radioactive territories. *Bioindication and radioactive contamination*. Nauka, Moscow, pp 213–217
- Yoschenko VI, Kashparov VA, Levchuk SE, Glukhovskiy AS, Khomutinin YV, Protsak VP, Lundin SM, Tschiersch J (2006a) Resuspension and redistribution of radionuclides during grassland and forest fires in the Chernobyl exclusion zone. Part II. Modeling the transport process. *J Environ Radioact* 87:260–278
- Yoschenko VI, Kashparov VA, Protsak VP, Lundin SM, Levchuk SE, Kadygib AM, Zvarich SI, Khomutinin YV, Maloshtan IM, Lashin VP, Kovtun MV, Tschiersch J (2006b) Resuspension and redistribution of radionuclides during grassland and forest fires in the Chernobyl exclusion zone. Part I. Fire experiments. *J Environ Radioact* 86:143–163
- Zymenko TG, Chernetsova IB, Mokhova SV (1995) Microbiologic complex in radioactively contaminated sod-potboil soils. *Her Nat Belarus Acad Sci (Biol)* 4:69–72