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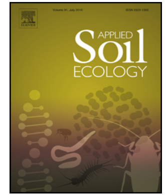

Effects of gamma irradiation on soil biological communities and C and N pools in a clay loam soil

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ABSTRACT

Gamma irradiation is becoming a promising technique in soil ecological studies because it has a particular advantage in selectively eliminating the target organism. But this selective sterilization technique is still in its initial exploratory stage and the subsequent impacts on soil carbon (C) and nitrogen (N) pools are relatively unknown for the majority of soils. Therefore, the responses of soil respiration, soil collembola, nematodes and microbial communities, and soil C and N pools (extracted with KCl, K_2SO_4 and H_2SO_4) to a range of gamma irradiation doses (0, 5, 10, 20 and 40 kGy) were determined under a clay loam soil in a 4-week incubation study. A flush of CO_2 was observed at the beginning of the incubation period (1–2 days) post-irradiation, and then strongly decreased relative to the unirradiated soils. At the middle of incubation period (11–14 days), there was a recovery of CO_2 efflux in the 5 kGy treatment. The effects of radiation on biological communities were dependent on taxa groups. The majority of collembola (>80%) and nematodes (>90%) were killed immediately in the higher doses (>5 kGy), but at lower doses of 5 kGy they were killed within 2 weeks after irradiation. The relative abundance of saprophytic fungi and protozoa decreased with increasing irradiation dose throughout the incubation period, while an opposite trend was found for some special bacterial taxa (19:1ω8c and i17:1ω9c). The resistance threshold of the entire microbial community to gamma irradiation was 10 kGy. The C and N contents in the KCl/K_2SO_4-extracted pools (except the dissolved organic C), in the H_2SO_4-extracted labile pool II (LP II) and in the recalcitrant pool (RP) decreased with increasing irradiation dose across the incubation period. The decreases in LP II and RP were accompanied by the increase in labile pool I. The variation in the level of all soil C and N pools was significantly affected by the radiation doses higher than 5 kGy. Our results indicate that the gamma dose between 5 and 10 kGy is sufficient to eliminate soil fauna without significant effect on microbial community compared to the unirradiated treatments. Moreover, a radiation dose of 5 kGy for selective defaunation has minor impacts on soil C and N pools of a clay loam soil. Our results also suggest that dose optimization is necessary due to high variability associated with irradiation levels effect on biological taxa in different soils.

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1. Introduction

Sterilized soil is often used to study soil biota and their function in biogeochemical cycle. The most commonly used sterilization techniques include steam autoclaving (Berns et al., 2008; Egli et al., 2006), freezing-thawing (Chelinbo et al., 2014; Hyvönen and Persson, 1996) and chemical agents (*e.g.* formaldehyde, methyl bromide and chloroform) (Trevors, 1996; Tuominen et al., 1994). But these techniques may cause irreversible changes in soil structure and chemical properties (Berns et al., 2008; Egli et al., 2006; Trevors, 1996). Irradiation is emerging as a promising technique because of its minimal soil disturbance (Bank et al., 2008; Berns et al., 2008; McLaren et al., 1962; McNamara et al., 2003; Trevors, 1996). It has been widely employed in the re-colonisation experiments (Buchan et al., 2013), soil enzyme

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activity (Knight and Dick., 2004; McLaren et al., 1962) and biodegradation experiments (Kelsey et al., 2010; Razavi darbar and Lakzian, 2007).

The sensitivity of soil organisms to gamma irradiation varies from species to species. The resistance threshold to gamma irradiation for most of the actinomycetes, fungi and invertebrates is around 10 kGy, and the majority of bacteria are eliminated at 20 kGy (Johnson and Osborne, 1964; McNamara et al., 2003; Voets et al., 1965; Zaitsev et al., 2014). This gives a possibility to selectively eliminate the target organism by changing the dose applied. Only one or two successful applications of this selective sterilization technique have been completed by Buchan et al. (2012, 2013), who recommended that 5 kGy is sufficient to eliminate soil nematodes and leave microbes intact. But, this dose is facing a tough challenge of reproducibility when it was applied to different soils or soil fauna groups (Gebremikael et al., 2015). Therefore, investigating the radio-sensitivity of soil organisms on a broader range of soil types would be beneficial to promoting the development of selective sterilization technique.

The release of nutrients from dead soil organisms, and the depolymerization of polysaccharide and lignin from solid organic matter after irradiation could result in changes in soil carbon (C) and nitrogen (N) pools (McNamara et al., 2003; Razavi darbar and Lakzian, 2007). These undesired changes may lead to an overestimation of the contribution of re-inoculated organism to soil C and N pools (Buchan et al., 2012; Gebremikael et al., 2015; McNamara et al., 2003). Although a few studies found a steep increase in the dissolved organic C (DOC) and N (DON) after irradiation (Buchan et al., 2012; Marschner and Bredow, 2002; Niedrée et al., 2012), there is still limited information about the changes in various soil C and N pools induced by irradiation.

In order to evaluate the impacts of gamma irradiation on soil microfauna and soil C and N pools, we conducted a microcosm experiment using a clay loam soil (Typic Hapludoll). Our objectives were: (1) to elucidate the impacts of a range of gamma doses on soil biotic communities (soil microbes, nematodes and collembola) and soil C and N pools (extracted with salt and acid solution), and (2) to find an optimal dose that could effectively eliminate soil fauna and maintain a comparable soil C and N pools and soil microbial community compared with unsterilized soils.

2. Materials and methods

2.1. Experimental design

The microcosm experiment was a completely randomized design with five treatments including: (1) non-irradiated (control, 0); (2) irradiated with 5 kGy (5); (3) irradiated with 10 kGy (10); (4) irradiated with 20 kGy (20); (5) irradiated with 40 kGy (40). Each treatment had five replications. The irradiation doses used in this experiment were similar to that of Buchan et al. (2012).

Microcosms consisted of PVC tubes (inner diameter 8 cm, height 15 cm) and were sealed at the ends with polyethylene (PE) films. Each tube was filled with 300 g homogeneous soil and gently compacted to a bulk density of 1.23 g cm^{-3}. The moisture content was adjusted to 50% water-filled pore space and the top of tube was sealed with Parafilm to prevent moisture loss. The soil used was collected from a long-term moldboard plow managed field (44°12′N, 125°33′E) in October 2014 (see Liang et al., 2007 for detailed description of this field trial). The soil is a typical Black soil (Typic Hapludoll, USDA Soil Taxonomy) with a clay loam texture (36.0% clay, 24.5% silt and 39.5% sand). The soil was sieved through a 5 mm mesh to remove coarse inorganic and organic material prior to filling.

A total of 75 microcosms (5 treatments × 5 replicates × 3 sampling times) were established and incubated at 25 °C for 9 days to allow the soils to equilibrate from the disturbance imposed by the experimental manipulations. After equilibration, all microcosms were transported to National Institute of Metrology (Beijing, China) and subjected to gamma irradiation with a dose of 5, 10, 20 or 40 kGy (^{60}Co Source with irradiation rate of 45.6 Gy min^{-1}). On return to the laboratory, the microcosms were kept in the dark at 25 °C for 4 weeks.

During the experiment, the microcosms were continuously aerated, and were moistened every four day with sterile ultrapure water to keep water at 50% water-filled pore space. Five microcosms from each treatment (control and irradiated) were destructively sampled at 0, 2 and 4 weeks of incubation.

2.2. Soil respiration

The CO_2 efflux was measured to estimate soil respiration rate using a Li-820 gas analyser (Li-Cor, Lincoln, Nebraska, USA) with the closed-flow chamber method at day 1, 2, 4, 6, 9, 11, 14, 18, 22 and 27 after incubation.

2.3. Soil biological community

Soil collembola and nematodes were extracted using the Macfadyen method (Macfadyen, 1961) and a modified cotton-wool filter method (Liang et al., 2009) respectively. These methods can effectively segregate the living organism from the dead one. The extracted organisms were preserved in 4% formalin for subsequent counting. The mortality of collembola or nematodes was calculated as the differences between control and irradiated treatments, and expressed as percentage.

Soil microbial community was characterized using phospholipid fatty acids (PLFAs) analysis as described by Bossio and Scow (1998) and Zhang et al. (2013). Lipids were extracted from 8 g of freeze-dried soil with a single-phase chloroform-methanol-citrate buffer (1:2:0.8) system and separated into neutral lipids, glycolipids, and polar lipids using solid phase extraction columns (Supelco Inc., Bellefonte, PA). The polar lipids were trans-esterified to the fatty acid methyl esters by a mild-alkali methanolysis. The resulting fatty acid methyl esters were redissolved in hexane containing 19:0 as an internal standard. They were analyzed using an Agilent 6850 series Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA).

The individual PLFAs i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0, i18:0, i15:1ω6c, i17:1ω9c represent gram-positive bacteria; 15:1ω7c, 16:1ω9c, 16:1ω7c, 17:1ω8c, 18:1ω7 c, 18:1ω6c, 18:1ω5c, 19:1ω8c, 20:1ω8c, 20:1ω9c, 16:0 2 OH, cy17:0ω7c, cy19:0ω7c, cy19:0ω9c represent gram-negative bacteria (Pietri and Brookes, 2009; Ding et al., 2015); 10 Me16:0, 10 Me17:1ω7c, 10 Me17:0, 10 Me18:1ω7 c, 10 Me18:0, 10 Me20:0 represent actinomycetes (Ding et al., 2015). The PLFA 16:1ω5 c was used as a measure of arbuscular mycorrhizae fungi (Bach et al., 2010; Zhang et al., 2013), and 18:1ω9c and 18:2ω6c were used to represent saprophytic fungi (Li et al., 2012; Dempsey et al., 2013). The PLFA 20:4ω6c was used as marker for protozoa (Vestal and White, 1989). Total PLFA was calculated by summing the concentrations of all identified PLFA (C14–C20), and was expressed as $nmol^{-1}$ dry soil. The ratio of cy/pre (cy17:0ω7c/16:1ω7c) was calculated to indicate nutritional or environmental stress (Helgason et al., 2010; Liang et al., 2012).

2.4. Salt-and acid-extractable soil C and N pools

Organic C and N pools were assessed in this study, including salt- and acid-extractable soil C and N pools. The dissolved organic C (DOC) was extracted with 2 M KCl (soil weight:solution volume = 1:5) for 1 h, and then filtered with a 0.45 μm membrane

and determined by a TOC analyzer (Model TOC-VCPH, Shimadzu, Tokyo, Japan). The microbial biomass C (MBC) and N (MBN) were extracted from the fumigated and non-fumigated soil samples with 0.5 M K_2SO_4 (Vance et al., 1987) and analyzed using a TOC analyzer (Model TOC-VCPH, Shimadzu, Tokyo, Japan). The MBC and MBN were calculated using extraction factors of 0.38 (Vance et al., 1987) and 0.54 (Brookes et al., 1985) respectively. K_2SO_4 extractable N in non-fumigated soil was used as dissolved organic N (DON) (Spedding et al., 2004).

The H_2SO_4-extractable organic C and N pools, including labile pool I C (LPI-C) and N (LPI-N), labile pool II C (LPII-C) and N (LPII-N), and recalcitrant C (RP-C) and N (RP-N), were studied following the protocol of Rovira and Vallejo (2002). Briefly, 50 mg soil was hydrolyzed with 20 mL of 2.5 M H_2SO_4 at 105 °C for 30 min in sealed Teflon tubes. The hydrolysate (labile pool I, LPI) was recovered by centrifugation and decantation. The remaining residue was further hydrolyzed with 2 mL of 13 M H_2SO_4 overnight at room temperature under continuous shaking. The suspension of the acid extractant was then diluted to 1 M with deionized water and the residue was hydrolyzed again for 3 h at 105 °C with occasional shaking. The hydrolysate (labile pool II, LPII) was recovered in the same manner as for the LPI. The residue was washed and dried, and taken as recalcitrant pool (RP). The C and N contents in LPI and LPII pools were analyzed with a TOC analyzer (Model TOC-LCPH, Shimadzu, Tokyo, Japan) and the C and N contents in RP pool with a FlashEA 1112 elemental analyser (ThermoFinnigan, Italy).

The LPI is known to predominantly contain polysaccharides which are of both plant origin (such as hemi-cellulose and starch) and microbial origin (mostly microbial cell walls) whereas the LPII is largely cellulose in composition. Lignin and lipid are usually dominant in the RP (Belay-Tedla et al., 2009; Rovira and Vallejo, 2002).

2.5. Data analysis

The following equation was used to calculate soil CO_2 emission rate: $F = (\alpha \times V/V_m)/M \times 10^{12}$, where F is CO_2 efflux from the soil (pmol g^{-1} s^{-1}), α is the mean rate of change of the CO_2 concentration in the chamber (ppm s^{-1}), V is the volume of chamber (m^3), V_m is the volume of 1 mol of an ideal gas (22.4 L mol^{-1} at STP), M is the dry mass of soil (g).

Prior to analysis, microbial abundance, dissolved organic carbon and microbial biomass carbon and nitrogen were ln ($x+1$) transformed to achieve normality. The effects of irradiation dose, incubation time and their interactions on the CO_2 emission rate, biological communities and soil C and N pools were analyzed using a two-way ANOVA. Partial eta-squared (PES) was used to describe the magnitude of the effect attributable to each factor. Differences among treatments were tested with Tukey's honestly significant difference ($P < 0.05$). To best summarize the variance caused by irradiation at the level of community, the data of microbial biomarkers and soil C and N pools were subjected to principal component analysis (PCA) respectively (van den Brink et al., 1996). Then the first axis sample scores were used to determine treatment effects by one-way ANOVA. All date analyses were performed with the software package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

To determine which species in the microbial community were affected most by gamma, the principal response curve (PRC) was carried out using CANOCO software 4.5 version (ter Braak, 1988). The PRC method is based on partial redundancy analysis (RDA). The PRC has the capacity to display the deviation of the treatment effects from the untreated control through time and to interpret the accompanying taxon weights at the species level. The taxon weights represent the affinity of each species to the treatments. The higher the weight, the more the actual response pattern of species is likely to follow the pattern in the PRC or *vice versa*. The Monte Carlo permutation tests (499 permutations) were run to determine the statistical significance of the first ordination axis.

3. Results

3.1. Soil respiration and biological community

Soil respiration was strongly affected by the factors of irradiation dose, incubation time and their interaction (Table 1, PES parameter, $P < 0.005$). On the first two days of incubation, the CO_2 efflux rate was significantly higher ($P < 0.05$) in the irradiated treatments than in the non-irradiated control with mean

Table 1
Influence of the two factors (irradiation treatments and incubation time) on biological properties and soil C and N pools.

		Treatment			Time			Treatment × Time		
		PES	*P*	F_4	PES	*P*	F_2	PES	*P*	F_8
Biological properties	CO_2 efflux rate	0.43	<0.001	36.231	0.86	<0.001	131.775	0.69	<0.001	12.145
	TPLFA	0.93	<0.001	151.430	0.24	0.002	7.202	0.67	<0.001	11.390
	Gram (+)	0.88	<0.001	84.883	0.29	0.001	8.994	0.55	<0.001	6.981
	Gram (−)	0.89	<0.001	90.871	0.49	<0.001	21.985	0.50	<0.001	5.616
	Actinomyc.	0.87	<0.001	71.921	0.24	0.002	6.927	0.38	0.004	3.418
	AMF	0.94	<0.001	186.872	0.51	<0.001	23.549	0.52	<0.001	6.156
	SF	0.95	<0.001	201.452	0.72	<0.001	57.051	0.71	<0.001	14.002
	Protozoa	0.64	<0.001	20.325	0.13	0.039	3.432	0.05	ns	0.295
	Cy/Pre	0.91	<0.001	114.794	0.81	<0.001	94.208	0.53	<0.001	6.224
C and N pools	DOC	0.96	<0.001	388.416	0.95	<0.001	614.353	0.62	<0.001	11.984
	DON	0.69	<0.001	33.587	0.85	<0.001	165.507	0.18	ns	2.728
	MBC	0.80	<0.001	61.116	0.20	0.018	7.281	0.16	ns	1.435
	MBN	0.77	<0.001	50.843	0.38	<0.001	18.637	0.22	ns	2.095
	LPI-C	0.74	<0.001	42.837	0.59	<0.001	43.055	0.41	<0.001	5.291
	LPII-C	0.62	<0.001	24.431	0.65	<0.001	56.092	0.18	ns	1.617
	RP-C	0.52	<0.001	16.067	0.57	<0.001	99.293	0.08	ns	0.683
	LPI-N	0.87	<0.001	104.242	0.05	ns	2.160	0.26	0.018	3.071
	LPII-N	0.66	<0.001	28.436	0.67	<0.001	61.757	0.31	0.003	3.296
	RP-N	0.63	<0.001	25.760	0.83	<0.001	148.007	0.21	ns	2.058

The partial eat squared (PES) describe the proportion of the variability attributable to a factor. For example, irradiation accounted for 45% of the variation in CO_2 efflux rate. TPLFA, total PLFA; G (+), Gram-positive bacteria; G (−), Gram-negative bacteria; Actinomyc., Actinomycetes; AMF, Arbuscular mycorrhizal fungi; SF, Saprophytic Fungi; Cy/Pre, cy17:0ω7c/16:1ω7c; LPI-C, labile C pools I; LPII-C, labile C pools II; RP-C recalcitrant C; LPI-N, labile N pools I; LPII-N, labile N pools II; RP-N recalcitrant N.

differences of 1.0–2.0, 2.3–3.5, 1.7–4.1 and 3.3–6.4 pmol g^{-1} s^{-1} in 5, 10, 20 and 40 kGy doses respectively (Fig. 1). However, this difference was reversed on day 4 with a steep decrease of CO_2 efflux in the irradiated treatments, especially in the 5 and 40 kGy. From the fourth day to the end of incubation, the rate of CO_2 efflux was significantly lower ($P < 0.05$) in the 20 and 40 kGy than in the 0 kGy with mean differences of 1.5 3.8 and 4.4 6.9 pmol g^{-1} s^{-1}, respectively. However, there was no significant difference between 0 and 5 kGy at the middle of incubation (11–14 days) (Fig. 1, $P > 0.05$).

The mortality of collembola and nematodes generally increased with increasing dose and incubation time (Table 2). At the beginning of incubation, low mortality (<80%) of collembola and nematodes was found in the irradiation dose lower than 10 kGy. However, after 2 weeks, this mortality was increased to 80% for collembola and 92% for nematodes (Table 2).

Both the factors (irradiation dose and incubation time) and their interaction had a significant effect on the abundance of TPLFA (Table 1, PES parameter, $P < 0.005$). During the entire incubation period, the abundance of TPLFA was lower in the 20 and 40 kGy than in the other treatments (Table 3, $P < 0.05$). A similar trend was also found for the other microbial functional groups, including gram-positive and gram-negative bacteria, actinomycetes, arbuscular mycorrhizae fungi and saprophytic fungi (Table 3, $P < 0.05$). The differences between the 0 and the 5 kGy and between the 5 and the 10 kGy were not significant for most functional groups especially at 2 weeks of incubation (Table 3). The abundance of protozoa was significantly influenced by irradiation dose and incubation time (Table 1, PES parameter, $P < 0.005$ and 0.05, respectively). The highest abundance was presented in the 0 kGy and the lowest in the 40 kGy across the entire incubation period (Table 3, $P < 0.05$). The ratio of cy/pre was significantly affected by the factors of irradiation dose, incubation time and their interaction (Table 1, PES parameter, $P < 0.005$). The lower value was observed in the control (0 kGy) than in the other treatments at the beginning of incubation; however, the cy/pre ratios were lower in the 5 and 10 kGy than in the 0 kGy at 2 and 4 weeks of incubation (Table 3, $P < 0.05$).

The results of principal response curve (PRC) analysis showed that a significant part of variation (34.6%) in the microbial community was explained by the first canonical axis of the PRC (Fig. 2, $P = 0.0022$). The biomarkers of soil saprophytic fungi (18:1ω9c and 18:2ω6c) and protozoa (20:4ω6c) relative to bacteria had lower negative taxon weight showing a sharp decline with increase in irradiation dose. For bacterial biomarkers, 19:1ω8c and i17:1ω9c showed the highest positive taxon weight and therefore an increase in relative abundance with increasing dose (Fig. 2).

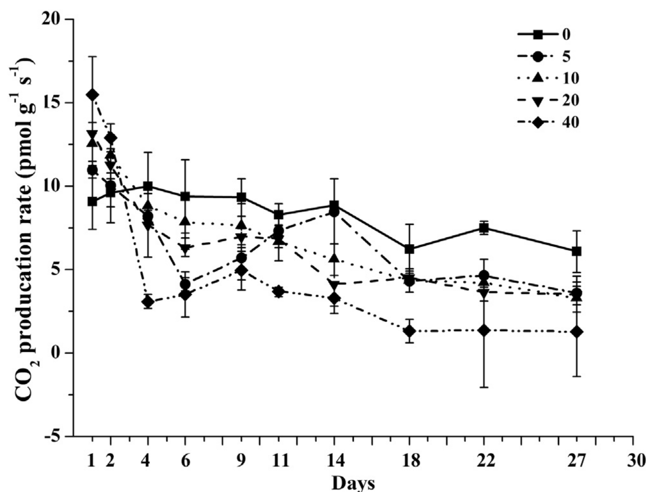

Fig. 1. Effects of gamma irradiation on soil respiration rate.

Table 2
The mortality of collembola and nematodes irradiated with different gamma doses.

	Treatment (kGy)	Time (weeks)		
		0	2	4
collembola (%)	5	69 ± 22	80 ± 45	96 ± 8
	10	75 ± 34	80 ± 45	100 ± 0
	20	77 ± 13	100 ± 0	100 ± 0
	40	100 ± 0	100 ± 0	100 ± 0
nematodes (%)	5	74 ± 23	92 ± 12	99 ± 2
	10	98 ± 3	100 ± 1	99 ± 2
	20	95 ± 5	100 ± 1	99 ± 2
	40	95 ± 3	100 ± 1	100 ± 0

The univariate analysis of the sample scores of microbial community along the first principal component revealed that the variance in the level of community was strongly affected by the irradiation dose at 20 and 40 kGy levels (Fig. 3a, $P < 0.05$).

3.2. Labile and recalcitrant soil C and N pools

The KCl- and K_2SO_4-extractable organic C and N pools were significantly affected by the irradiation dose and incubation time factors (Table 1, PES parameter, $P < 0.05$). Interactive effects between irradiation dose and time were only observed for DOC (Table 1, PES parameter, $P < 0.005$). The contents of DOC increased with increasing irradiati on dose in all incubation time (Fig. 4a). Compared with the control (0 kGy), the dose of 5 kGy significantly increased the DOC (1.5-fold) at first 2 weeks, but the other doses strongly increased the DOC during the entire incubation ($P < 0.05$; 1.2–1.7, 1.4–2.3 and 2.7–3.0-fold for 10, 20 and 40 kGy doses, respectively). DON, MBC and MBN tended to decrease with the increase in irradiation dose regardless of the incubation time, with the lowest values in the 40 kGy (Fig. 4b–d, $P < 0.05$). The highest MBC and MBN contents were presented at the beginning of incubation in all treatments, while the highest DOC and DON were observed at week 2 ($P < 0.05$).

The factors of irradiation dose and incubation time had a significant effect on the H_2SO_4-extractable organic C and N pools (Table 1, PES parameter, $P < 0.005$). The interactions of irradiation dose and incubation time were significant for LPI-C, LPI-N and LPII-N (Table 1, PES parameter, $P < 0.05$). The contents of LPI-C and LPI-N increased with increasing dose during the entire incubation. The higher values were associated with the 20 and 40 kGy treatments than with the 0 kGy (Table 4, $P < 0.05$). However, an opposite trend was found for LPII-N. The contents of LPII-C, RP-C and RP-N generally decreased with increasing irradiation dose regardless of the incubation time (Table 4). Furthermore, their contents were lower in the 20 and 40 kGy than in the other treatments (Table 4, $P < 0.05$).

The univariate analysis of the sample scores of all soil C and N pools along the first principal component showed that the variance in the level of community was strongly affected by the doses higher than 5 kGy (Fig. 3b, $P < 0.05$).

4. Discussion

4.1. Changes in soil biotic community

Soil respiration is a useful indicator in measuring the changes in soil biological activity (Anderson, 1982). In this study, a flush of CO_2

Table 3
Effects of gamma irradiation on the abundance (mean ± SD) of total PLFA and selected biomarkers for major microbial groups.

Time (Weeks)	Treatment (kGy)	nmol^{-1} dry soil							
		TPLFA	Gram (+)	Gram (−)	Actinomyc.	AMF	SF	Protozoa	Cy/Pre
0	0	21.89 ± 0.74a	6.30 ± 0.16a	4.75 ± 0.34a	3.59 ± 0.17a	0.39 ± 0.03a	1.43 ± 0.11a	0.29 ± 0.05a	1.06 ± 0.01d
	5	19.72 ± 0.81b	5.76 ± 0.26b	4.53 ± 0.25a	3.17 ± 0.02b	0.31 ± 0.02b	1.24 ± 0.03b	0.23 ± 0.02a	1.09 ± 0.02 cd
	10	20.14 ± 0.49b	5.89 ± 0.08b	4.61 ± 0.20a	3.25 ± 0.15ab	0.28 ± 0.01b	1.12 ± 0.02b	0.21 ± 0.04a	1.14 ± 0.02c
	20	15.66 ± 0.81c	4.75 ± 0.31c	3.70 ± 0.14b	2.65 ± 0.19c	0.23 ± 0.02c	0.69 ± 0.06c	0.15 ± 0.00 ab	1.26 ± 0.05b
	40	15.10 ± 0.93c	4.58 ± 0.32c	3.21 ± 0.27b	2.44 ± 0.19c	0.20 ± 0.01c	0.60 ± 0.05c	0.10 ± 0.12b	1.33 ± 0.03a
2	0	19.23 ± 0.62a	5.59 ± 0.23a	4.20 ± 0.08a	3.13 ± 0.15a	0.34 ± 0.01a	1.17 ± 0.03a	0.28 ± 0.03a	1.22 ± 0.02b
	5	20.05 ± 0.61a	5.74 ± 0.29a	4.42 ± 0.29a	3.07 ± 0.14a	0.27 ± 0.02b	1.02 ± 0.07ab	0.19 ± 0.03b	1.09 ± 0.01d
	10	20.35 ± 0.71a	5.98 ± 0.18a	4.65 ± 0.23a	3.22 ± 0.16a	0.28 ± 0.01b	0.95 ± 0.03b	0.19 ± 0.03b	1.16 ± 0.02c
	20	15.16 ± 0.91b	4.61 ± 0.31b	3.33 ± 0.21b	2.61 ± 0.24b	0.22 ± 0.02c	0.66 ± 0.10c	0.14 ± 0.01bc	1.32 ± 0.02a
	40	13.62 ± 0.93b	4.01 ± 0.30c	2.82 ± 0.31c	2.21 ± 0.08c	0.16 ± 0.03d	0.52 ± 0.10c	0.08 ± 0.09c	1.35 ± 0.03a
4	0	19.94 ± 0.15a	5.69 ± 0.17ab	4.10 ± 0.15a	3.39 ± 0.10a	0.37 ± 0.01a	3.39 ± 0.10a	0.25 ± 0.03a	1.29 ± 0.03b
	5	19.15 ± 0.52ab	6.11 ± 0.31a	3.91 ± 0.15a	3.11 ± 0.18ab	0.26 ± 0.02b	3.11 ± 0.18b	0.19 ± 0.01ab	1.20 ± 0.02c
	10	18.27 ± 0.19b	5.58 ± 0.11b	3.95 ± 0.19a	3.01 ± 0.15b	0.23 ± 0.02bc	3.01 ± 0.15b	0.18 ± 0.12ab	1.25 ± 0.02b
	20	16.69 ± 0.60c	4.96 ± 0.18c	3.22 ± 0.02b	2.91 ± 0.21b	0.19 ± 0.01d	2.91 ± 0.21bc	0.11 ± 0.12ab	1.45 ± 0.08a
	40	16.58 ± 0.79c	5.09 ± 0.29c	3.36 ± 0.22b	2.54 ± 0.13c	0.22 ± 0.02 cd	2.54 ± 0.13c	0.00 ± 0.00b	1.38 ± 0.05ab

TPLFA, total PLFA; G (+), Gram-positive bacteria; G (−), Gram-negative bacteria; Actinomyc., Actinomycetes; AMF, Arbuscular mycorrhizal fungi; SF, Saprophytic Fungi; Cy/Pre, cy17:0ω7c/16:1ω7c; ns, no statistical significance at the $P = 0.05$ level; degrees of freedom were 2, 4 and 8 for time, treatment and time × treatment, respectively.

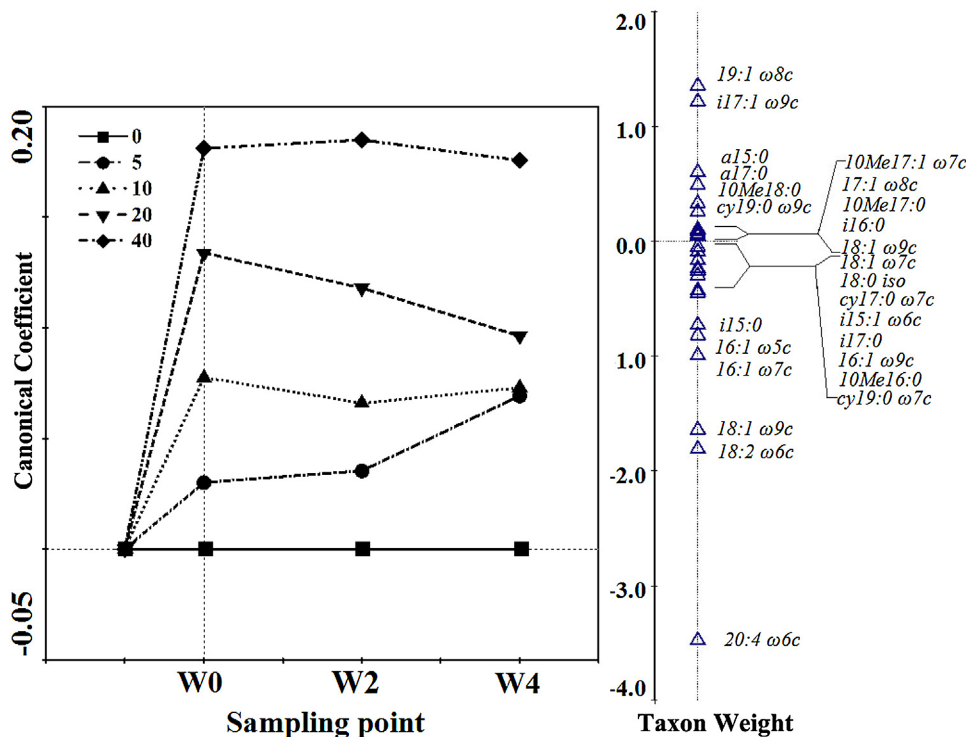

Fig. 2. Principal Response Curve (PRC) for the effects of gamma irradiation on the microbial biomarkers. Presented is the canonical coefficient of the different irradiation doses at the sampling points (0, 2 and 4 weeks) and the taxon weight for the major biomarkers. A positive weight can be interpreted as a positive move away from the untreated control of the particular biomarkers when the treatment also has a positive canonical coefficient. The proportion of variability explained by the first response curves was 34.6% (*Eigenvalue* = 0.222, *F-Ratio* = 31.777, $P = 0.0022$).

emission was observed within the first 1–2 days of incubation post-irradiation, and then substantively decreased relative to the control (Fig. 1). Our observations agree well with the results obtained by Cawse and Mableson (1971), which ascribed this initial flush to the decarboxylation of organic matter and the subsequent decline to the death of organism. However, there was an obvious recovery of CO_2 efflux in the 5 kGy treatment at the middle of incubation period (Fig. 1). Considering no litter was added as the available substrate in this study and a high abundance of TPLFA was maintained in the 5 kGy treatment at 2 weeks of incubation (Table 2), this recovery was primarily from the surviving microbes which could restore their activity with the environment of higher available nutrient and energy resources and less competition in the irradiated soils (McNamara et al., 2007; Niedrée et al., 2013).

As a general rule, the greater the size of an organism the more the sensitivity to radiation (Edwards, 1969; McNamara et al., 2003; Zaitsev et al., 2014). It is possible to infer that the number of dying collembola would be greater than that of nematodes at the same irradiation dose. However, in the present study, we did not observe this trend (Table 2). This difference might have been diluted by the

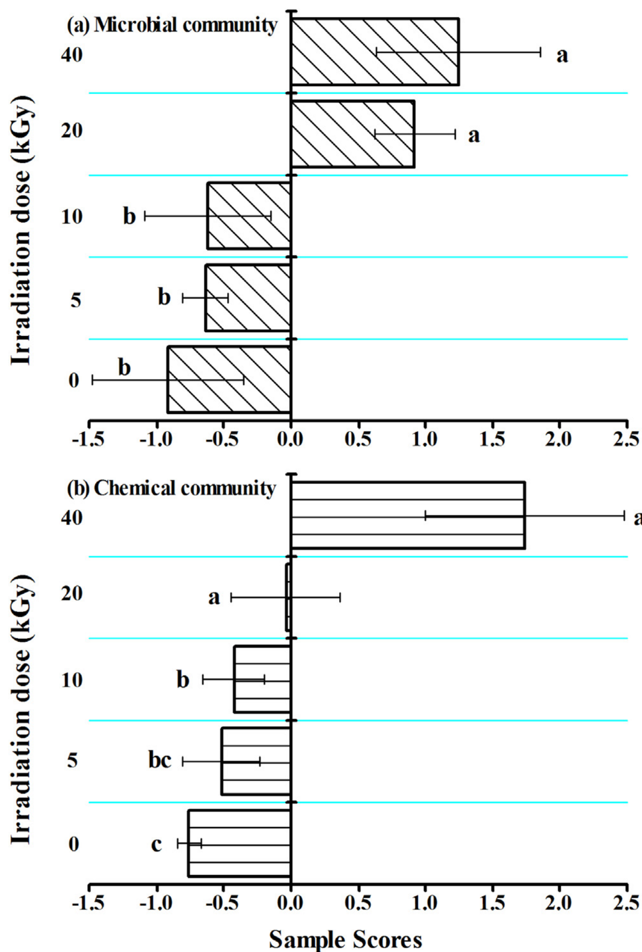

Fig. 3. Effects of gamma irradiation on the sample scores of the first principal component of microbial (a) and chemical (b) community.

high variability of the mortality of collembola (Table 2). The increased patchiness distribution of collembola induced by irradiation (Krivolutsky et al., 1992; Zaitsev et al., 2014) makes it difficult to collect a uniform (representative) subsample.

Radiation has a dose-dependent lethal effect on soil biota (Fuller et al., 2015; Gebremikael et al., 2015; McNamara et al., 2003; Zaitsev et al., 2014). Both the mortality of collembola and nematodes and the number of dying protozoa increased with increasing irradiation dose (Tables 2 and 3), but this effect appears weak on most soil microbial functional groups. There was no significant difference between the 0 and 5, 10 kGy doses in TPLFA, gram-positive and gram-negative bacteria and actinomycetes at 2 weeks of incubation (Table 3). This might be due to their higher tolerance to ionizing radiation than soil fauna (McNamara et al., 2003; Zaitsev et al., 2014).

Buchan et al. (2012) suggested that the interpretation of the absolute abundance changes based on PLFA profiles irradiated by gamma irradiation should be applied with caution because the PLFAs still persisted when the MBC was greatly reduced or eliminated. Therefore, in our study, we focused on evaluating the relative abundance not the absolute abundance. The relative abundance of saprophytic fungi was reduced more strongly than that of bacteria (Fig. 2), which might be due to the higher sensitivity of saprophytic fungi to irradiation than bacteria (McNamara et al., 2007; Zaitsev et al., 2014). Among the biomarkers of bacteria, both the relative abundance of gram-negative biomarker 19:1ω8c and gram-positive biomarker i17:1ω9c increased with increasing irradiation dose (Fig. 2). This might have resulted from the different reproductive strategies of the two bacterial groups. The 19:1ω8c, which belongs to the *r*-strategists, can quickly colonize and grow under the elevated labile organic C conditions induced by radiation; while the i17:1ω9c, belonging to the *k*-strategists, tend to change physiologically in response to the stress (Jin et al., 2014; Schurig et al., 2013; Zaitsev et al., 2014).

There was no significant difference among the 0, 5 and 10 kGy doses at the level of microbial community (Fig. 3a), suggesting that

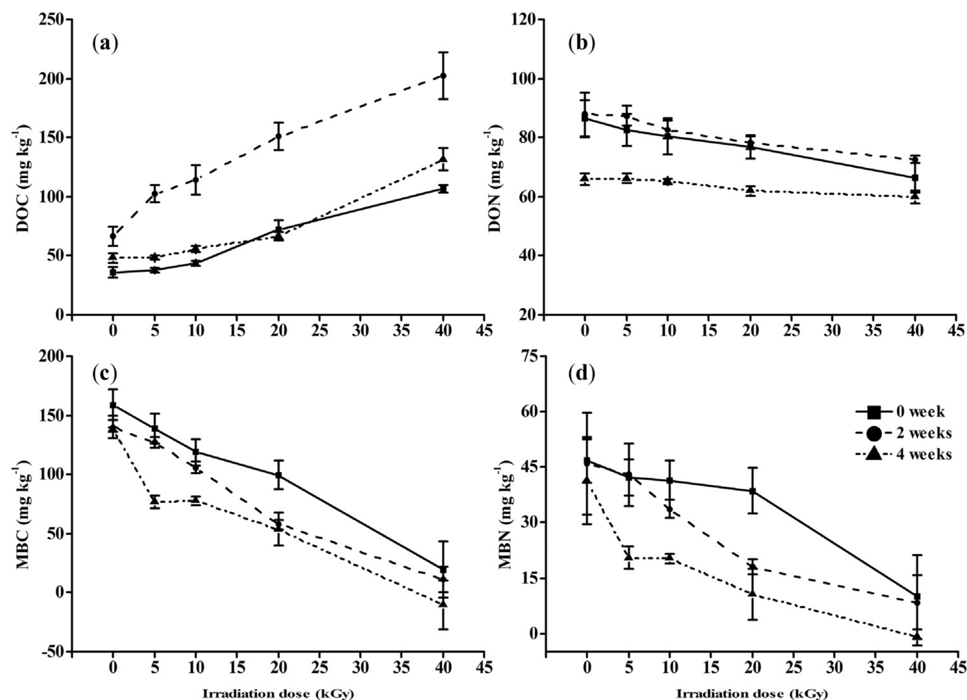

Fig. 4. Effects of gamma irradiation on soil dissolved organic C (DOC, a) and N (DON, b), microbial biomass C (MBC, c) and N pools (MBN, d).

Table 4
Effects of gamma irradiation on the concentrations (mean ± SD) of labile, recalcitrant C and N pools.

Time (Weeks)	Treatment (kGy)	mg kg^{-1} dry soil					
		LPI-C	LPII-C	RP-C	LPI-N	LPII-N	RP-N
0	0	2923 ± 112bc	5412 ± 95a	13339 ± 541a	823 ± 7c	1451 ± 72a	737 ± 37a
	5	2781 ± 245c	4780 ± 260b	13130 ± 592b	841 ± 37b	1262 ± 87ab	695 ± 32b
	10	2852 ± 71bc	4178 ± 52c	13153 ± 415ab	871 ± 48b	1202 ± 81b	718 ± 24ab
	20	3050 ± 99b	4009 ± 303c	12421 ± 265c	878 ± 23ab	1192 ± 52b	647 ± 22c
	40	3441 ± 285a	3550 ± 148d	12600 ± 638c	1010 ± 6a	1147 ± 25c	630 ± 20c
2	0	2940 ± 257c	4407 ± 44a	12524 ± 729a	842 ± 38b	1270 ± 18a	654 ± 54ab
	5	3032 ± 101bc	3568 ± 46b	12624 ± 339a	847 ± 14b	1243 ± 34ab	677 ± 26a
	10	3080 ± 370bc	3456 ± 61bc	12241 ± 387a	873 ± 11b	1218 ± 35b	623 ± 18bc
	20	3303 ± 224b	3473 ± 341bc	11627 ± 144a	887 ± 51b	1198 ± 47b	619 ± 26bc
	40	3651 ± 205a	3318 ± 78c	11547 ± 673a	1020 ± 57a	1057 ± 53c	574 ± 44c
4	0	3040 ± 153c	3788 ± 180a	11397 ± 197a	847 ± 45c	1138 ± 102a	562 ± 10ab
	5	3138 ± 482c	3317 ± 207a	11640 ± 311a	872 ± 25b	1120 ± 40a	585 ± 26a
	10	3309 ± 186c	3258 ± 441a	11535 ± 395a	877 ± 9b	1080 ± 55ab	560 ± 19b
	20	4007 ± 164b	3233 ± 829a	10788 ± 333b	903 ± 30ab	1002 ± 63b	526 ± 18c
	40	4569 ± 143a	3045 ± 345a	10393 ± 147b	1118 ± 8a	990 ± 75b	490 ± 21d

LPI-C, labile C pools I; LPII-C, labile C pools II; RP-C recalcitrant C; LPI-N, labile N pools I; LPII-N, labile N pools II; RP-N recalcitrant N; ns, no statistical significance at the $P = 0.05$ level; degrees of freedom were 2, 4 and 8 for time, treatment and time × treatment, respectively.

the resistance threshold of the entire microbial community to gamma irradiation was 10 kGy. The threshold value 10 kGy is much higher than that of 5 kGy as also reported by Buchan et al. (2012, 2013) and 3 kGy by Gebremikael et al. (2015). These differences might arise from the contents of soil moisture and organic matter, and soil texture (Gebremikael et al., 2015; McNamara et al., 2003). Because the soil moisture was adjusted to 50% water-filled pore-space both in our study and in the experiment of Buchan et al. (2013), the relatively high soil organic matter contents in clay-textured soils (17.56 g kg^{-1} organic C) *vs.* in sandy-textured soils (10.3–12.1 g kg^{-1} organic C) may determine the differences in threshold values. The soil with higher contents of clay and soil organic matter may shield more microorganisms from irradiation (McNamara et al., 2003), and therefore the level of resistance arises higher.

A time-lag effect of radiation on soil biota is expected due to the delayed mutagenic and cytogenetic effects (Fuller et al., 2015; Little et al., 1997; Szumiel, 2015). This was revealed from the soil fauna data (Table 2). For example, the majority (>90%) of nematodes died after 2 weeks incubation with the 5 kGy dose. Popenoe and Eno (1962) and Buchan et al. (2012) also pointed out that 14 days were required for completely removing all free living nematodes in the low irradiated doses (≤5 kGy). In contrast, at the same time, the abundance of TPLFA in the 5 kGy was equal to the level of that in unirradiated soils (Table 3). This suggested that the nutrients release from dead soil fauna can be utilized by the surviving microbe after irradiation. Combining the results of lower stress indicator (expressed as Cy/Pre ratio) observed in the 5 kGy treatment at 2 weeks (Table 2), it is reasonable to speculate that these released substrates would achieve a maximum peak at this time.

4.2. Changes in labile and recalcitrant soil C and N pools

The increase in labile pool I, including LPI-C and LPI-N, was accompanied by the decrease in labile pool II and recalcitrant pool after irradiation with gamma (Table 4), suggesting that the cellulose in labile pool II and the lignin and lipid in recalcitrant pool can be decomposed into small polymers in a clay loam soil. Berns et al. (2008) also observed the strong losses of carbohydrate and N-alkyl region from solid organic matter in a sandy loam and a silt loam soils after gamma irradiation based on nuclear magnetic resonance data. These results indicate that larger soil organic molecule can be broken down into smaller fragments after exposure to ionizing radiation across different soil types, because ionizing radiation can create free hydrogen and hydroxyl radicals that are reactive as reducing and oxidizing agents to cleave C—C and N—N bonds (Bank et al., 2008; Razavi darbar and Lakzian, 2007; Wardman, 2009).

The decomposition from solid organic matter and the nutrient release from dead soil organisms may contribute to the increase in DOC after irradiation (Fig. 4a). However, the changes in DON content did not exhibit the similar trend as DOC but similar with the MBC and MBN, which was strongly decreased in the irradiated treatments relative to the non-irradiated control (Fig. 4 b–d). This might be primarily due to the fact that the impaired and depressed biological activity induced by gamma irradiation cannot maintain the generation of DON (Neff et al., 2003; Seely and Lajtha, 1997). The above presented results clearly suggest that the distribution of soil C and N pools could be modified by gamma irradiation. Furthermore, the magnitude of the modification was increased with the increase in irradiation dose. However, the modification induced by a 5 kGy irradiation could be neglected because there was no significant difference in chemical community between the 0 and the 5 kGy treatments (Fig. 3a).

Unlike other soil C and N pools, which had the highest value at the initial or the end of incubation period after radiation, DOC and DON reached to a highest value at 2 weeks of incubation (Fig. 4a,b). This might be due to a lag phase in the released organic matter originated from killed soil biota (Shaw et al., 1999).

5. Conclusions

The abundance of soil organisms and the concentration of soil C and N pools were significantly changed with gamma irradiation in a clay loam soil. The dose-dependent effect of irradiation was observed in soil C and N pools, and in soil collembola, nematodes and protozoa communities, but not in soil microbial community. This might be because some bacterial taxa have high tolerance to ionizing radiation, such as 19:1ω8c and i17:1ω9c. When the gamma irradiation dose lies in the range between 5 and 10 kGy, the majority of collembola and nematodes can be effectively eliminated without significant effect on the entire soil microbial community. To maintain a comparable soil C and N pools between sterilized and unsterilized soils, 5 kGy is an appropriate dose for selective defaunation. But this elimination would take 2 weeks to

complete. Thus, we propose that a 14 day pre-incubation after radiation in a clay loam soil is needed for evaluating the contribution of re-inoculated organism to soil C and N pools, especially to DOC and DON.

This research also suggests that evaluation of the response of soil biological and chemical properties to irradiation is necessary prior to employing selective sterilization technique by gamma irradiation, because the optimal dose will vary with faunal groups and soil types.

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