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Short communication

Selective sterilisation of undisturbed soil cores by gamma irradiation: Effects on free-living nematodes, microbial community and nitrogen dynamics

David Buchan*, Bram Moeskops, Nele Ameloot, Stefaan De Neve, Steven Sleutel

Department of Soil Management, Faculty of Bioscience Engineering, University of Ghent, Coupure Links 653, 9000 Ghent, Belgium

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ABSTRACT

The techniques available for sterilisation or defaunation of soil in ecological experiments mostly have strongly unwanted effects on soil structure and the dynamics of major nutrients such as nitrogen. The potential for using gamma irradiation to prepare defaunated soil microcosms was investigated by subjecting undisturbed soil cores to a range of irradiation doses (0, 5, 10, 20 and 40 kGy). The absence of living nematodes at the lowest irradiation dose was confirmed by microscopic observation. The effects of irradiation dose on mineral nitrogen (as NO_3^- and NH_4^+), microbial biomass C (C_{mic}), and phospholipid fatty acid (PLFA) concentration and composition were determined over a 4 week incubation period. An increase in the concentration of NO_3^- occurred during the incubation period after exposure to 0, 5 and 10 kGy but was barely detectable at 20 and 40 kGy. The effect of irradiation dose on NH_4^+ release was complex and highly variable within treatments, with the 10 kGy dose resulting in the highest concentrations. Microbial biomass carbon was significantly reduced following a 20 kGy irradiation dose and below detection at 40 kGy. Most remarkably, the sum of all measured PLFAs did not differ significantly between most treatments and was not correlated with microbial biomass. In most cases the concentration of signature fatty acids only differed significantly between the control and the highest irradiation dose treatments. To ascertain the sensitivity of microbial taxa to acute gamma irradiation with accuracy, measures of microbial community structure other than PLFA analysis are needed.

Experiments in soil ecology rely on manipulating the abundance, distribution or diversity of one or several groups of soil biota. Ecological theories are often tested on sterilised soil re-inoculated with filtered microbial slurries assumed to be representative of the original microbial community (e.g., Jones et al., 1998). Few sterilisation techniques are sufficiently selective not to affect non-targeted biota or the physical and chemical condition of soil. Chemicals such as methyl bromide, formaldehyde and mercuric chloride are efficient inhibitors of microbial activity (Tuominen et al., 1994); however they contaminate soil with toxic residues, making re-inoculation problematic. Autoclaving is a widespread sterilisation technique but has the disadvantage of causing high nutrient release and loss of soil structure (Trevors, 1996). Defaunation methods such as freezing also modify the physical structure and pore network of soil. Given the important feedbacks between soil biota and the physical heterogeneity of the soil habitat, the development of representative soil microcosms has been proposed as a key experimental innovation required for further understanding the activity of soil biota (Crawley et al., 2005).

Gamma irradiation allows the selective elimination of soil organisms by varying the dose applied, either directly by cell lysis or indirectly through the formation of mutagenic free radicals (McNamara et al., 2003). Because it leaves soil structure intact and devoid of any residual toxicity, it is an attractive technique for ecological experiments with undisturbed soil. Gamma irradiation also increases nutrient availability – in particular nitrogen – although the effects thereof vary considerably as a function of the applied dose (Lensi et al., 1991). An irradiation dose of 25 kGy is considered to sterilize soil, which has traditionally been verified by the absence of culturable bacteria. However, microscopic observation has revealed the persistence of dead cells (Ramsay and Bawden, 1983) and culture-independent methods for assessing the microbial community in chemically perturbed soil are now recognized to be superior to plate-culture methods (Kozdrój and van Elsas, 2001; Ritz, 2007).

Among soil fauna, free-living nematodes occupy a central role in the soil food web and contribute substantially to nutrient cycling (Verhoef and Brussaard, 1990). Although we found no studies on

* Corresponding author. Tel.: +32 9 264 60 66; fax: +32 9 264 62 47.
E-mail addresses: david.buchan@ugent.be (D. Buchan), bram.moeskops@ugent.be (B. Moeskops), n.ameloot@ugent.be (N. Ameloot), stefaan.deneve@ugent.be (S. De Neve), steven.sleutel@ugent.be (S. Sleutel).

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the response of entire nematode populations to gamma irradiation, studies on individual species indicate a survival threshold between 4 and 6 kGy (Chinnasri et al., 1997; McNamara et al., 2003; Thompson, 1990).

We investigated the effect of gamma irradiation on mineral nitrogen, nematode abundance, and soil microbial biomass and community structure. Soil cores were subjected to a range of biologically relevant irradiation doses based on McNamara et al. (2003) with the following expected outcomes: 5 kGy (no nematodes), 10 kGy (no fungi); 20 kGy (only bacteria) and 40 kGy (no microbial biomass at all).

Undisturbed cores (PVC tubes, 5 × 5 cm) were collected from a sandy loam soil (38% silt, 7% clay, bulk density 1.44 Mg m^{-3}, organic C 0.84%) on an organically managed trial field under fallow (ILVO, Merelbeke, Belgium) in March 2008. Cores were randomly assigned to one of five following treatments: control (not exposed to irradiation) or a dose of 5, 10, 20 or 40 kGy of gamma irradiation (^{60}Co source with irradiation rate of 11.1 kGy h^{-1}, Sterigenics, Fleurus, Belgium). All treatments were destructively sampled in triplicate after 0 (initial parameters), 2 and 4 weeks of incubation at 18 °C. Free-living nematodes were counted in the 0 and 5 kGy treatments only after 0 and 2 weeks using the zonal centrifuge extraction method (Hendrickx, 1995) to separate free-living nematodes from other soil constituents based on their specific density. Mineral N (NO_3^- and NH_4^+) was measured colorimetrically by continuous flow and microbial biomass carbon (C_{mic}) was determined using the fumigation-extraction technique (Vance et al., 1987) using a k_{EC} value of 0.45 (Joergensen, 1996). The organic carbon extracted in non-fumigated duplicates $C_{K_2SO_4}$ was considered as a separate variable. Phospholipid fatty acids (PLFAs) were extracted from freeze-dried soil using a modified Bligh and Dyer (1959) technique fully described in Moeskops et al. (2010). Only FAMES present in proportions of more than 1% of the total ($n = 49$) were retained for further analysis and summed to give 'total PLFA' ($n = 25$). Taxon-specific markers were taken from Moeskops et al. (2010) except that due to co-elution of 18:2ω6,9c and *cy*19:0, we used 18:1ω9c as an alternative fungal biomarker (Joergensen and Wichern, 2008) and only *cy*17:0 as an indicator for Gram-negative bacteria.

All variables except nematode abundance were subjected to two-way ANOVA, with *irradiation dose* (5 levels) and *incubation time* (3 levels) as factors; posthoc multiple pair-wise comparisons were carried out using Tukey's HSD method. Mineral nitrogen, C_{mic} and $C_{K_2SO_4}$ data were log $(X + 1)$ transformed prior to analysis to meet assumptions of normality and homoscedasticity.

Nematodes seemed initially unaffected by irradiation (8.8 ± 2.5 nematodes g^{-1} in 0 kGy versus 10.4 ± 3.1 nematodes g^{-1} in 5 kGy), however after 2 weeks their abundance was twice as low in 5 kGy compared to 0 kGy (4.3 nematodes g^{-1} and 9.4 nematodes g^{-1} respectively from composite samples). None of the nematodes counted in 5 kGy were in motion, whereas in 0 kGy more than 90% of nematodes were clearly active and mobile. Further microscopic investigation revealed that in 5 kGy the majority (>90%) of the nematodes were highly degraded and devoid of visible body contents. Although a 5 kGy dose was clearly sufficient to kill all nematodes, the extraction technique we employed did not separate dead from living nematodes. The remains of irradiated nematodes were not rapidly decomposed in soil, which was also observed for testate amoeba by Coûteaux (1992) who attributed this delay to the release of more easily available sources of C than the remains of dead soil fauna.

Gamma irradiation had a strong effect on NO_3^- and NH_4^+ ($p < 0.0001$ for both variables), however the duration of incubation only had a significant effect on NO_3^- ($p < 0.0001$, Fig. 1). At higher doses, NO_3^- concentrations were either very low (20 kGy) or below

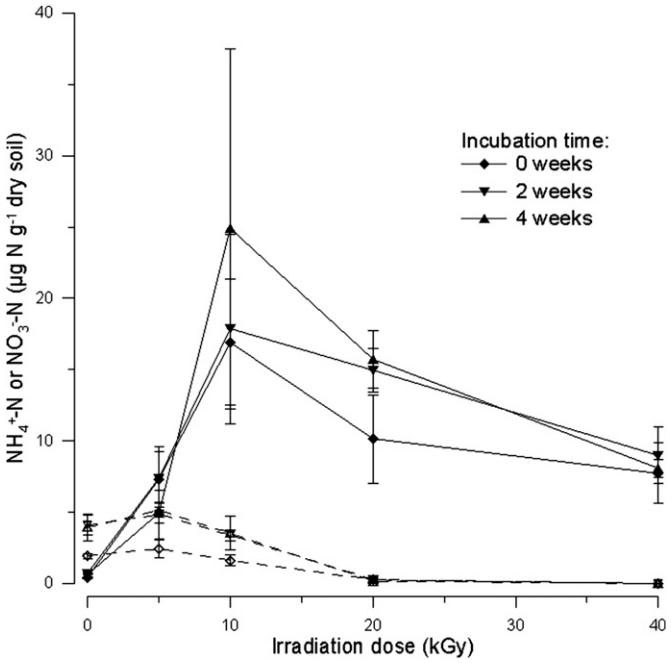

Fig. 1. Effect of gamma irradiation dose on NO_3^-–N (dotted lines) and NH_4^+–N (full lines).

the detection limit (40 kGy). The concentration of NH_4^+ showed a liner increase from ca. 1 µg N g^{-1} in 0 kGy to a maximum of 20–30 µg N g^{-1} in 10 kGy, and decreasing with higher irradiation doses to 8–12 µg N g^{-1} in 40 kGy (Fig. 1). Decreased NO_3^- concentrations beyond 10 kGy and a concomitant increase in NH_4^+ have been documented previously and are due to the depression of nitrifying bacteria (Lensi et al., 1991). Increased NH_4^+ concentrations and the suppression of nitrification were also reported by De Neve et al. (2004) following the application of fumigants to soil. The relatively high release of NH_4^+ in 10 kGy is explained by rapid

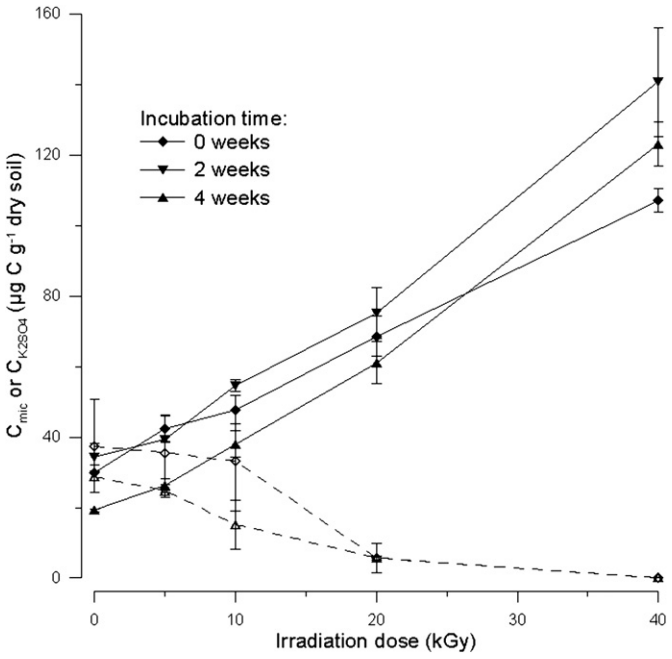

Fig. 2. Effect of gamma irradiation dose on microbial biomass C (dotted lines) and K_2SO_4-extractable organic C (full lines).