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The effect of varying gamma irradiation doses and soil moisture content on nematodes, the microbial communities and mineral nitrogen

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ABSTRACT

Gamma irradiation is becoming a potential tool in soil ecological studies as it gives a possibility to vary a dose that selectively kills the target organism. It makes minimum changes in soil biochemical properties as compared to autoclaving and freezing. Although gamma irradiation has often been used in studying the roles of nematodes on nutrient cycling, the recommended doses to eliminate nematodes still lead to a nutrient flush, and are less reproducible. Given that the indirect effect of gamma irradiation is through radiolysis, the same dose might have a different effect on the soil biochemical properties as the soil moisture content changes. Thus, an optimal dose that eliminates nematodes needs to be determined taking the moisture content of the soil sample at the time of irradiation into consideration. We conducted an incubation experiment for about three months during which the effects of a range of low gamma irradiation doses (0, 1, 3 and 5 kGy) at different moisture content (air dried, 50% water filled pore space (WFPS), 80%WFPS) were tested on nematode abundance and selected soil biochemical properties. Leaching with water immediately after irradiation at 50%WFPS, was used to assess to what extent it would remove N from the flush. The results showed that as the moisture content of the soil increases from 50%WFPS to 80%WFPS, nematode abundance, enzyme activities and total mineral N concentrations decreased during most of the incubation period at each irradiation dose. Increasing the moisture content, however, did not make significant changes in total phospholipid fatty acid concentrations in all levels of irradiations except at 3 kGy. These findings indicate the effects of gamma irradiation on soil biological and chemical properties vary depending on the moisture content of the soil at the time irradiation. Moreover, the moisture content of the soil at the time of gamma irradiation need to be considered during optimizing gamma irradiation as a tool in selective sterilization or defaunation. In the current experimental set up, the application of 3 kGy at 80%WFPS at the time of irradiation eradicated nematodes and left a comparable microbial abundance and community structure to the unirradiated CTR. Leaching the 50% WFPS samples irradiated at 5 kGy dose immediately after irradiation generally significantly reduced both NH_4^+-N and NO_3^--N concentrations, but NH^+_4-N concentration remained higher compared to the unirradiated CTR. This indicates that leaching with water only partly reduced the nutrient flush and underlines the need to test additional methods to reduce the nutrient flush further.

1. Introduction

Nematodes are among the most numerous multicellular soil organisms and possess key positions in the soil food web (Bongers and Bongers, 1998; Ferris, 2010 Yeates, 2003). They also show diversified feeding strategies that enable them to interact intensively with plants, microbes and other nematodes and thereby significantly contribute to nutrient cycling (de Ruiter et al., 1994; Gebremikael et al., 2014; Ingham et al., 1985).

The contributions of these nematodes to nutrient cycling have been often quantified by comparing simple differences in nutrient dynamics between completely sterilized samples reinoculated with nematodes and without nematodes (Chen and Ferris, 1999; Ingham et al., 1985; Xiao et al., 2010). The drawback to this straightforward approach, however, is that sterilized or defaunated samples, usually, exhibit significant differences in biological,

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chemical and physical properties as compared to untreated fresh soil. Findings based on such disturbed soils may not be representative and more difficult to extrapolate to actual conditions occurring in the field.

Realistic defaunating methods for the investigation of the contribution of nematodes to nutrient cycling should completely eliminate nematodes without causing significant alterations to other biological and chemical properties, particularly the microbial communities and nutrient concentrations. At present, however, there is no sterilizing and/or defaunating technique that does not impact the microbial communities and nutrient concentrations. For example, one of the most widely used technique in investigating the roles of nematodes in nutrient cycling, which is autoclaving (Ingham et al., 1985; Xiao et al., 2010), completely eradicates all the biota, produces a high nutrient flush and changes the physical properties of the soil (Alphei and Scheu, 1993; Powlson and Jenkinson, 1976). After autoclaving, reinoculating the microflora is mandatory, but it has proven very difficult to reconstruct the microbial community, both in terms of abundance and community structure. Moreover, the reincoulated microflora do not occupy the same spatial position compared to the indigenous microbes (Recorbet et al., 1995), which may lead to over grazing by the soil fauna (Bonkowski and Clarholm, 2012; Xiao et al., 2010). The other common method is freezing and thawing for varying frequencies which appears to be effective in defaunating soil samples (Djigal et al., 2004). However, it significantly changes the physical properties and more importantly the biological properties such as an increase in microbial respiration following the decomposition of ruptured or dead microbial cells (Deluca et al., 1992). Other techniques such as heating in the range of 40–60 °C have been reported to selectively kill nematodes from soil samples, however, the possible nutrient flushes and changes in microbial biomass and composition were not measured directly (Jaffee, 2006).

Another technique that has often been used in soil ecology studies and recently in nematode studies is gamma irradiation. It is emerging as a potential and a preferable defaunating method because of two main reasons (McNamara et al., 2003): (1) the possibility to vary doses to selectively kill nematodes while leaving the microbial community largely intact and (2) its lesser effect on soil chemical and physical characteristics particularly at lower doses (Lensi et al., 1991; Lotrario et al., 1995; Salonius et al., 1967). Several studies have been conducted to investigate the response of nematodes to various gamma irradiation doses ranging from 1 to 40 kGy (Buchan et al., 2012; Singh and Kanehiro, 1970; Thompson et al., 1990).

Despite the advantages of using gamma irradiation a number of concerns remain, such as nutrient flushes during and after irradiation and the reproducibility of the method. The nutrient flush during irradiation is mainly due to degradation of the organic matter (McLaren, 1969; McNamara et al., 2003; Popenoe and Eno, 1962). The presence of unaffected microbes and extracellular enzymes which degrade the killed microbial biomass are responsible for the release of nutrients after gamma irradiation (Shih and Souza, 1978). The problem with such nutrient flush is that the activity of the reinoculated target organism may be significantly enhanced by this soluble C and N concentration resulting in an overestimation of their contribution as compared to the control.

The lower reproducibility of the method could be mainly due to moisture content differences at the time of irradiation that controls the indirect effects of gamma irradiation. The indirect effect is due to radiolysis of water, that is, the water inside and surrounding the cell dissociates in to free oxygen, hydrogen and hydroxide radicals, which can damage the DNA (Aquino, 2012; McNamara et al., 2003). As a result, the same dose may have different lethal effects depending on the moisture content of the soil at the time of irradiation.

Accordingly, we hypothesized that (i) the killing effect of a given dose of gamma irradiation increases as the moisture content of the soil increases at the time of irradiation, particularly at lower doses. (ii) This increased lethal effect could result in the release of more dissolved organic compounds due to the rupture of the killed cells that may eventually result in an increase in nutrient flush. Reducing these dissolved organic compounds by leaching with water after gamma irradiation may decrease the nutrient flush after irradiation.

To test these hypotheses, we applied a range of low gamma irradiation doses on soil samples with varying moisture contents (air dried, 50%WFPS and 80%WFPS), and did incubation experiment for about 3 months during which we determined and compared the effect of each dose-moisture combination on microbial biomass and community structure, enzymatic activities, nitrogen mineralization and nematode abundances. Moreover, we studied the extent that leaching with water immediately after gamma irradiation reduces N flush.

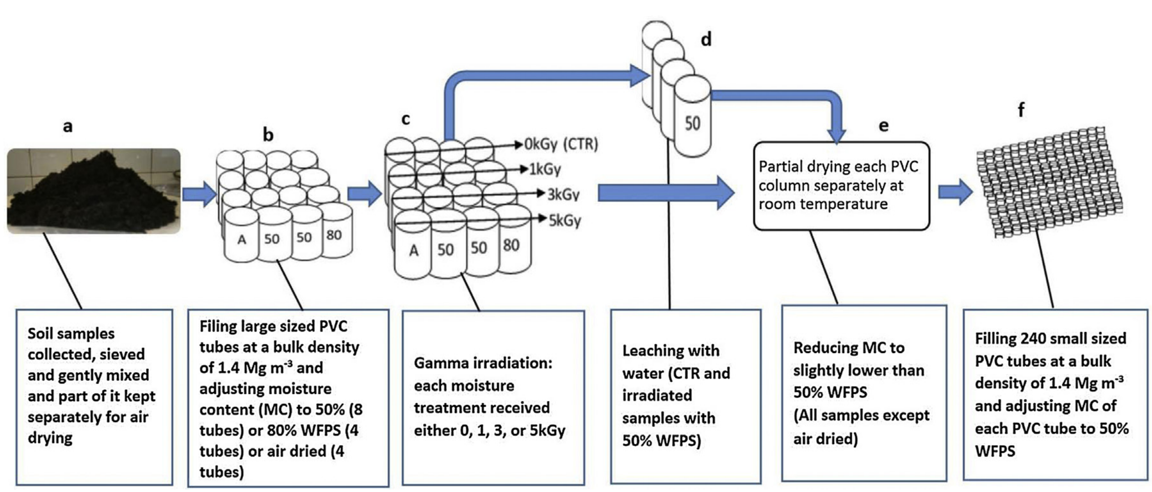

Fig. 1. Schematic representation of the experimental set up. Soil samples were collected, sieved and mixed to make a homogenous bulk sample on which manipulations were done to obtain different experimental treatments.