Soil Biology & Biochemistry 60 (2013) 142–155

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The effect of free-living nematodes on nitrogen mineralisation in undisturbed and disturbed soil cores

David Buchan*, Mesfin Tsegaye Gebremikael, Nele Ameloot, Steven Sleutel, Stefaan De Neve

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

ARTICLE INFO

Article history:
Received 12 April 2012
Received in revised form
24 January 2013
Accepted 26 January 2013
Available online 13 February 2013

Keywords:
Microbial biomass
Nematodes
Gamma irradiation
Phospholipid fatty acids
Undisturbed cores
Nitrogen mineralisation

ABSTRACT

Soil fauna, particularly nematodes, are considered to strongly contribute to nitrogen mineralisation through grazing on microflora during decomposition. Demonstration of this effect has mostly relied on calculation-based soil food web analyses or experiments involving simplified and artificially constructed food webs. We carried out an incubation experiment in which defaunated soil cores were reinoculated with entire soil nematode populations extracted from bulk soil and during which nitrogen mineralisation was measured. Both undisturbed and disturbed cores were prepared to investigate whether a representative pore structure influences the effect of entire free-living nematode populations on nitrogen mineralisation. Cores were subjected to a 5 kGy gamma irradiation dose sufficient to eliminate all soil fauna while leaving the microbial biomass largely intact. Half of the irradiated cores were reinoculated with nematodes extracted from a corresponding volume of bulk soil and incubated for 82 days. The microbial biomass was not strongly affected by gamma irradiation or nematode addition but declined strongly in all treatments during incubation. Reinoculation of nematodes was successful in establishing populations of a similar size and composition as in the control samples. Net nitrogen mineralisation from indigenous soil organic matter was observed in all treatments throughout the incubation, but was always more pronounced in irradiated cores. Total mineral nitrogen concentrations did not differ significantly between simply irradiated and irradiated then reinoculated cores. However by the end of the incubation period nematode addition resulted in 87% and 23% more NO_3^-–N g^{-1} dry soil in undisturbed and disturbed cores respectively, while NH_4^+–N g^{-1} dry soil decreased by 50% in both core types. We found no convincing evidence for a contribution of free-living nematodes on total nitrogen mineralisation, but the activity of nitrifying organisms was clearly stimulated by nematode grazing.

1. Introduction

Soil fauna have been estimated to collectively contribute to approximately 30% of nitrogen mineralization (Verhoef and Brussaard, 1990) by excreting excess N in the form of ammonium and by regulating the size and activity of the microbial community. Microbivorous nematodes contribute strongly to this process by occupying key trophic positions in the soil food web. Soil free-living nematodes include bacterivorous and fungivorous nematodes that directly influence nitrogen mineralisation and microbial turnover (Hunt et al., 1987), as well as omnivorous and predatory nematodes which may indirectly influence nitrogen mineralisation by regulating the population of microbivorous nematodes (Wardle et al., 1995).

This contribution of nematodes to nitrogen mineralisation has previously been investigated by means of two distinct approaches: empirically determined in additive microcosm experiments (Anderson and Ineson, 1982; Woods et al., 1982; Ingham et al., 1985; Griffiths, 1986; Bruckner et al., 1995; Ferris et al., 1998; Bardgett and Chan, 1999) or theoretically derived from soil food web models (e.g. Hunt et al., 1987; Brussaard et al., 1990; Moore and de Ruiter, 1991). Although food web models are based on field measurements for the estimation of the biomass of the different functional groups, they are sensitive to a number of uncertain input parameters such as the C:N ratios of bacteria and their substrates (De Ruiter et al., 1993) and the assimilation and production efficiencies of various faunal groups (Verhoef and Brussaard, 1990). Lastly food web models do not explicitly take account of indirect contributions of soil fauna to N mineralisation through the modification of factors limiting microbial growth (Beare et al., 1992).

* Corresponding author. Tel.: +32 9 264 60 66; fax: +32 9 264 62 47.
E-mail addresses: david.buchan@ugent.be, ecolodave@gmail.com (D. Buchan), mesfintsegaye.gebremikael@ugent.be (M.T. Gebremikael), n.ameloot@ugent.be (N. Ameloot), steven.sleutel@ugent.be (S. Sleutel), stefaan.deneve@ugent.be (S. De Neve).

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.01.022

The use of microcosms to study the influence of soil fauna on ecosystem processes such as nitrogen mineralisation has seen numerous improvements and developments in complexity over the decades (Huhta, 2007). However, besides a clear bias of most microcosm studies towards small volumes and relatively short incubation times (Kampichler et al., 2001), most microcosm experiments have lacked realism compared to natural soil in at least one of the following three ways: (1) a modified soil physical structure due to soil pre-treatment effects such as sterilisation; (2) a reinoculated microbial community of highly reduced or uncharacterised diversity; (3) an artificially assembled soil food web with often a single species of fauna representing a functional group.

Despite proven links between processes carried out by soil biota and the physical heterogeneity of the soil habitat (Crawley et al., 2005), the establishment of a representative soil pore structure has seldom received attention in microcosm studies. However in a series of experiments dealing with the dynamics of nematode populations in response to changes in matric potentials, undisturbed cores were used but nematode populations were not explicitly manipulated in a controlled manner (Görres et al., 1999; Neher et al., 1999). In a similar type of experiment, Yeates et al. (2002) first defaunated undisturbed cores and then reinoculated them with the bacterivorous nematodes *Cephalobus*, *Pristionchus* and *Rhabditis* to study the effects of matric potential on population dynamics and feeding activity.

To prepare microcosms, soil is usually first mixed, sieved and repacked; occasionally soil is also dried and re-wetted prior to incubation. In some cases a structurally simple and homogenous medium such as acid-washed sand has been used instead of soil (Ferris et al., 1998; Chen and Ferris, 1999). Common sterilisation procedures such as autoclaving have been shown to modify the soil pore structure (Clarholm, 1985). Kampichler et al. (1999) showed that defaunation by freezing allowed intact soil monoliths to be used as experimental units without much physical disruption; however the effect of freezing on microflora was not studied. Gamma irradiation is one technique that can be used to either sterilise or selectively remove soil fauna from soil without affecting its physical structure (McNamara et al., 2003; Buchan et al., 2012). The influences of such pre-treatments on processes of interest in microcosm experiments have, to the best of our knowledge, not been systematically studied yet.

A controlled biotic community is mostly established by complete sterilisation followed by reinoculation with a single or handful of micro-organisms easily cultured in laboratory environments at the worst (Coleman et al., 1978; Anderson et al., 1981; Griffiths, 1986; Chen and Ferris, 1999), or a microbial slurry filtered from a soil suspension at the best (Clarholm, 1985; Jones et al., 1998; Sulkava and Huhta, 1998; Bardgett and Chan, 1999; Xiao et al., 2010). Except for one study where microbial communities reinoculated into sterile soil were compared to the controls by PLFA analysis (Griffiths et al., 2008), we found no attempts to compare the composition or size of reinoculated microbial communities with the original ones in undisturbed soil in field conditions.

There has been a long tradition in microcosm experiments to test ecological theories using an artificially constructed soil food web with a select amount of species, especially in the case of nematodes (Woods et al., 1982; Ingham et al., 1985; Griffiths, 1986). Yet related species of nematodes with similar feeding habits have been shown to differ in their effect on nitrogen mineralisation (Ferris et al., 1998). In a few controlled experiments, nematode species from trophic groups other than bacterivores and fungivores have been used to test the effect of omnivory in a simple soil food web (Mikola and Setälä, 1999). We found no studies including predatory and/or omnivorous dorylaimid nematodes, presumably because these are not easily cultivated in laboratory media. Free-living root-feeding nematodes such as Tylenchidae, which in many soils make up a significant part of the nematode community, are also not commonly included in experimental studies of nitrogen mineralisation. Because of these limitations, potentially important ecological processes such as interspecific competition or predation have been overlooked. In addition there is a risk of making generalised conclusions concerning the relationship between functional diversity and an ecosystem process, when in fact species-specific effects are being observed (Mikola, 1998). However some experiments have been conducted using whole nematode communities extracted from soil (Sulkava and Huhta, 1998) or derived from soil and intentionally reared so as to be dominated by bacterivores (Xiao et al., 2010).

To address the issues highlighted above we chose to carry out an incubation study using both undisturbed and disturbed (i.e. sieved and homogenised) soil cores, in which we attempted to leave the microflora intact and only selectively remove soil fauna using non-disruptive low-dose gamma irradiation. Most importantly, we chose to reinoculate nematode populations extracted directly from soil into our defaunated microcosms. We made no use of amendments and instead focused on mineralisation of indigenous soil organic matter over a longer time period.

The aims of this study were to assess whether entire nematode populations in microcosms caused increased nitrogen mineralisation compared to defaunated microcosms, and whether this influence differed between undisturbed and disturbed microcosms. Two secondary aims relating to the methodology we employed were to verify whether the microbial community differed between control and irradiated microcosms, and whether reinoculated nematode populations differed from those in control microcosms.

2. Materials and methods

2.1. Field sampling and core preparation

Undisturbed cores and bulk soil were collected in October 2008 from an organic agriculture trial field (ILVO, Merelbeke, Belgium) on which clover had been sown earlier in the spring. The soil had a sandy loam texture, and at sampling the pH_{KCl} averaged 5.3, organic C content was 1.03% and C:N ratio was 11. Due to poor establishment of the clover, it was possible to collect bare, plant-free soil for this experiment. Six undisturbed samples were collected to determine bulk density (1230 ± 27 Mg m^{-3}) and field moisture content (0.165 ± 0.002 g g^{-1}) in the 0–10 cm layer within a ca. 10×10 m area at least 5 m from the edges of the field. From this same area 54 *undisturbed cores* were collected from the upper 7.5 cm by inserting bevelled PVC tubes (height 7.5 cm, diameter 7.5 cm). These were capped on both ends and stored with minimal physical disturbance. A large amount of bulk soil was also collected from the same area and depth layer. Bulk soil was first mixed in buckets and coarsely sieved (10 mm mesh) to remove stones and root fragments and then further gently homogenised. Bulk soil was used to refill another 54 *disturbed cores* with 280.0 g fresh soil, which was gently compacted to the same bulk density as measured in the field. The remaining bulk soil was stored in black plastic bags in a basement at around 17 °C until nematode extraction (no more than two days later).

2.2. Gamma irradiation and nematode reinoculation

One third ($n = 18$) of both type of cores were set aside as control cores and kept overnight in a basement at ca. 17 °C along with the remaining bulk soil. All the other cores were subjected to gamma irradiation at a dose of 5 kGy (Sterigenics industrial facility, Fleurus, Belgium), following a previously determined