Interactions between free-living nematodes, microbes and plants: effects on soil nutrient cycling, microbial properties and plant nutrient uptake

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Dutch translation of the title:

Interacties tussen vrijgevende nematoden, de microbiële gemeenschap en planten: effecten op de nutriëntendynamiek in de bodem, microbiële eigenschappen en de opname van nutriënten door planten

To refer this thesis:


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Gent, Belgium
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<th>Description</th>
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<tbody>
<tr>
<td>AIRDRIED</td>
<td>Air dried samples (treatment)</td>
</tr>
<tr>
<td>AMF</td>
<td>Arbuscular mycorrhizal fungi</td>
</tr>
<tr>
<td>B:F ratio</td>
<td>Bacterial: fungal ratio (PLFAs)</td>
</tr>
<tr>
<td>BI</td>
<td>Basal index</td>
</tr>
<tr>
<td>CI</td>
<td>Channel index</td>
</tr>
<tr>
<td>C&lt;sub&gt;mic&lt;/sub&gt;</td>
<td>Microbial biomass carbon</td>
</tr>
<tr>
<td>c-p</td>
<td>Coloniser-persister</td>
</tr>
<tr>
<td>CTR</td>
<td>Control (treatment)</td>
</tr>
<tr>
<td>Cum C</td>
<td>Cumulative C mineralized</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved organic matter</td>
</tr>
<tr>
<td>EI</td>
<td>Enrichment index</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectroscopy</td>
</tr>
<tr>
<td>kGy</td>
<td>kiloGray, SI unit of gamma irradiation</td>
</tr>
<tr>
<td>FAME</td>
<td>Fatty acid methyl ester</td>
</tr>
<tr>
<td>IC</td>
<td>Ion chromatography</td>
</tr>
<tr>
<td>Leached</td>
<td>Soil samples leached with water after gamma irradiation</td>
</tr>
<tr>
<td>MI</td>
<td>Maturity index</td>
</tr>
<tr>
<td>MI 2-5</td>
<td>Maturity index for c-p classes 2-5</td>
</tr>
<tr>
<td>+Nem</td>
<td>irradiated samples reinoculated with nematodes (treatment)</td>
</tr>
<tr>
<td>-Nem</td>
<td>irradiated samples reinoculated with nematodes (treatment)</td>
</tr>
<tr>
<td>OM</td>
<td>Organic matter</td>
</tr>
<tr>
<td>Pi</td>
<td>Inorganic phosphorus (water extractable)</td>
</tr>
<tr>
<td>PLFA</td>
<td>Phospholipid fatty acid</td>
</tr>
<tr>
<td>PPI</td>
<td>Plant-parasitic index</td>
</tr>
<tr>
<td>PPN</td>
<td>Plant parasitic nematodes</td>
</tr>
<tr>
<td>PNP</td>
<td>Para Nitro Phenol</td>
</tr>
<tr>
<td>SI</td>
<td>Structure index</td>
</tr>
<tr>
<td>SIM</td>
<td>Single ion measurement (GC-MS measurement method)</td>
</tr>
<tr>
<td>SOM</td>
<td>Soil organic matter</td>
</tr>
<tr>
<td>TIC</td>
<td>Total ion count (GC-MS measurement method)</td>
</tr>
<tr>
<td>TTC</td>
<td>Triphenyl tetrazolium chloride</td>
</tr>
<tr>
<td>TPF</td>
<td>Triphenyl formazan</td>
</tr>
<tr>
<td>UNA</td>
<td>Unamended</td>
</tr>
<tr>
<td>WFPS</td>
<td>Water-filled pore space</td>
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Chapter 1:

Introduction
1.1 Soil biodiversity and roles in ecosystem functioning

An enormous number and diversity of organisms live in soil, interacting with each other and with their physical environment. Several important ecosystem functions and services are regulated by these biotic and abiotic interactions. Knowledge on the soil biodiversity for instance, the number of species present in a particular ecosystem may not be often directly correlated with their function. The soil biodiversity has been classified mainly using size classes and functional classes as an effort in quantifying their role in ecosystem functions. The most common way of size based classification groups the soil organisms into four size classes according to the body width (Swift et al., 1979). The two smallest groups (<100 µm body width) include the microflora such as bacteria, fungi and archeae, and microfauna such as protozoa and nematodes. The remaining soil animals are categorized as mesofauna (between 100 µm and 2 mm) such as mites and collemboela, and macrofauna (>2 mm) such as earthworms, enchytraeids and myriapods. The other classification of soil biodiversity is functional in which groups of species with similar traits and which perform similar functions are put into the same functional group. The main criteria in this case are the feeding habit and life history traits such as energy utilization, reproductive and turnover rates (Moore and Hunt, 1998; Yeates et al., 1993b). The functional groups in soil ecology often categorize bacteria and fungi as primary decomposers, plant feeding nematodes as herbivores, bacterivorous nematodes and protozoa and fungivorous nematodes as microbial grazers, collemboela and mites as predators, and earthworms and ants as ecosystem engineers (Brussaard, 1998; Hunt et al., 1987). Turbé et al. (2010) categorized the soil biota into three major functional groups namely: chemical transformers (bacteria and fungi), biological regulators (nematodes, protozoa, collemboela and enchytraeids) and ecosystem engineers such as earthworms.

Kibblewhite et al. (2008) categorize ecosystem functions of the soil biota into four aggregate groups namely, I) carbon transformation, II) nutrient cycling, III) soil structural maintenance and IV) biological population regulation. Carbon transformation is one of the fundamental processes in the soil ecosystem and involves every group of organism in the soil. Although between 80-99% of the heterotrophic respiration is carried out by bacteria and fungi, soil animals play a great role in enhancing C transformation through shredding the residues and
dispersing microbes (Persson et al., 1980). These soil animals such as mites, millipedes, earthworms and termites that feed on detritus and facilitate C transformation and nutrient cycling are also called decomposers. Microbial grazers such as nematodes and protozoa also play an important role in nutrient cycling by regulating the microbial activity and turnover. Other specific groups of organisms such as AMF and N₂ fixing bacteria are involved in nutrient cycling, in addition to the vast diversified groups of decomposers that are involved in C transformation. The role of soil organisms in nutrient cycling is crucial for primary production and water quality.

The mesofauna and macrofauna such as mites and earthworms are mostly involved in the soil structure maintenance function. They create channels, pores and aggregates that profoundly influence gas and water transport in the soil (Brussaard et al., 1997). Microbes are also involved in this process of soil structure maintenance by producing organic compounds that play significant roles in soil aggregation. The function of biological population regulation is carried out by predators, microbivorous and hyper parasites including organisms recognized as pests and diseases of agriculturally important plants and animals as well as humans. Each of the above mentioned crucial ecosystem functions is a result of interactions between diversified groups of organisms. In an effort to link the ecosystem services to society values Brussaard et al. (2007), estimated more than 1.5 trillion US dollars per year for services provided by the soil biota worldwide, based on available reports. Because the topic of this thesis is about the interactions between soil organisms and their role in nutrient cycling, the remaining sections focus on the below ground multitrophic interactions in general and between microbes, nematodes and plants in particular.

1.2 Multitrophic below ground interactions and nutrient dynamics

Energy and nutrients enter the soil system from several sources such as plants, added organic residues and artificial fertilizers. Soil organisms primarily derive energy and nutrients for their metabolic needs from the soil organic matter. OM dynamics and nutrient cycling in soil are regulated by multitrophic interactions between functionally dissimilar soil organisms across all size classes and trophic levels. The soil food web diagram by de Ruiter et al. (1993) depicts belowground multitrophic interactions and the resulting energy and nutrient flows through
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each functional group of organisms (Figure 1.1). Following the incorporation of detritus into the soil system, different organisms start decomposing the organic matter. Energy and the major nutrients C, N, P and S pass through each compartment as the process of mineralization and immobilization proceeds during the course of OM decomposition.

Figure 1.1. A soil food web model of a winter wheat field under integrated farming management in The Netherlands (de Ruiter et al., 1993). The red, orange and green colored arrows represent the bacterial, fungal and root channels respectively.

The smallest size classes and the primary decomposers (bacteria and fungi) in the first decomposer trophic level perform an extremely wide range of chemical transformations through catabolic and anabolic reactions. Most of the soil bacterial communities are heterotrophic, i.e. acquire C, energy and other nutrients for their metabolic activities by decomposing organic matter found in different forms in soil. These bacterial communities release appropriate enzymes that facilitate the decomposition of a particular organic matter pool. This decomposition results in the release of C, H₂O and major nutrients such as N, P and S. Other bacteria such as Rhizobia establish symbiotic relationships with plants to fulfil their C
needs. Rhizobia in return fix N from the atmosphere and make it available for the plants in the form of NH$_4^+$ that can be directly assimilated. Non symbiotic plants grown mixed with legumes also benefit from such symbiotic relationship as N fixed by the rhizobia becomes available for uptake (Bardgett et al., 1999a). Fungi are heterotrophic organisms that use a large variety of organic substrates ranging from simple C compounds such as glucose to the very complex ones such as lignin. Some of the fungi directly interact with plants and facilitate nutrient availabilities in two main ways: through pathogenic activity (e.g. *Fusarium spp*) and mutualistic relationship (e.g. mycorrhiza). During the symbiotic associations, the mycorrhizal fungi constantly utilize the carbohydrates produced by plants during photosynthesis. The fungi in turn enable the plants to use the large surface area of the networked filaments that grow in and around the plant to gain access to minerals and water that could not be reached by plants otherwise. Microfauna may enhance the AMF growth as they make N available to AMF by grazing bacteria. This has been shown for protozoa (Koller et al., 2013c). Nematodes may also enhance such mutualistic relationship between plants and AMF, as they release N like the protozoa.

The microfauna (nematodes and protozoa) and mesofauna (collembola and mites) greatly interact with bacteria and fungi. Given that nematodes and protozoa are extremely abundant and require a minimum water film for survival, they generally occupy the same habitat and can easily access microbes that live in smaller pore spaces (Bouwman et al., 1994). Faunal grazing makes nutrients available for plants that would otherwise have been locked up in the microbial biomass. Particularly, microbivorous protozoa and nematodes play a significant role in this regard. Food web models estimated that bacterial feeding nematodes and protozoa contribute 83% of the total N mineralization by the soil fauna. In some forest ecosystems where bacteria are found to cause net N immobilization, soil fauna may contribute more than fungi to N mineralization (Osler and Sommerkorn, 2007). The mesofauna such as collembola and mites have been shown to contribute to nutrient cycling primarily indirectly through strong comminution effects (grinding the litter) and extensive grazing of microbial tissue (Kaneda and Kaneko, 2007; Seastedt, 1984; Vreeken-Buijs et al., 1998). Kaneda and Kaneko (2007) reported that Collembola may excrete high nutritional substrate that stimulate bacteria and fungi, which in turn stimulates the activities of microbial feeding nematodes.
Bardgett and Chan (1999) have shown that interactions between collembola and nematodes enhance N mineralization in montane grass land ecosystems. Predators and omnivorous faunal groups positioned at the higher level of the soil food web also contribute to nutrient cycling and OM decomposition indirectly through regulating the abundances of microbial grazers. For instance, mites feed on important nematode groups such as bacterivorous and herbivorous nematodes and indirectly regulating the primary decomposers (Brussaard et al., 1997; Verhoef and Brussaard, 1990).

Macrofauna such as earthworms, enchytraeids and termites mainly feed on a range of organic matter forms suggesting that they show limited trophic relationships (Turbé et al., 2010). As the functional name given to them (ecosystem engineers) indicates, they influence OM dynamics indirectly by modifying or creating habitats that facilitates the movement of water and gas, or by regulating the availability of resources for other species (Jones et al., 1994). However, it has been shown that soil organisms such as fungi, protozoa, algae and nematodes may constitute a significant part of the diet of earthworms, indicating direct trophic relationships (Monroy et al., 2008; Tao et al., 2011). Decomposition of residues generally involves a succession of different faunal groups as the decomposition proceeds. For instance, decomposition process of wheat residues in a litter bag was dominated by bacterivorous nematodes and bacterivorous and nematophagous mites at the initial decomposition stage (VreekenBuijs and Brussaard, 1996). However, the later stage was dominated by fungivorous nematodes, fungivorous and omnivorous mites and collembola and predatory mites indicating that the rate of decomposition is predator controlled. Tao et al. (2011) also showed earthworms decreased the abundance of nematodes and enchytraeids.

Plants are an integral part of the belowground multitrophic interactions as all soil organisms depend on plants directly or indirectly for their C need. It has been suggested that the amount of roots present in the soil can be as large as, or even larger than, the amount of aboveground plant biomass (Turbé et al., 2010). It has also long been recognized that many interactions between microbes, soil fauna and plants do occur in soils that control microbial activity, nutrients availability and plant growth. For example, a small infestation by root feeding nematodes triggered production of root exudates that stimulated the growth of microbes in the soil, which in turn increased the growth of their grazers (mainly protozoa and nematodes)
and eventually increased nutrient availabilities. Such nutritional effects of interactions between microbes, fauna (particularly protozoa and nematodes) and plants has been presented as a microbial loop (Clarholm, 1985) (Figure 1.2). The suggested interaction effect is that plants release root exudates with high C: N (Nguyen, 2003), which fuels bacterial growth and stimulates them to mineralize N from the native organic matter to fulfil their additional N need associated with the additional C assimilated. The protozoa/nematodes then consume the increased bacterial biomass and release part of the N into the soil that in turn becomes available for plant uptake and increased plant growth. Clarholm (1985) found that the presence of protozoa increased plant N uptake in sterilized microcosms reinoculated with bacteria by 75% compared to similar samples without protozoa.

Plants would also benefit not only from the enhanced availability of nutrients, but also from root proliferation and increased production of growth hormones in the rhizosphere as a result of fauna-microbe interactions. Mao et al. (2007) found that the presence of bacterial feeding nematodes increased the root growth and structure of tomato plants accompanied by the increase of soil indolyl-3-acetic acid (IAA) content. Belowground plant-nematode interactions can also affect above ground plant performance and competition that may result in plant community structure changes (Van der Putten et al., 2001; Wardle et al., 2004).
Figure 1.2. A conceptual model for possible interactions between microbes, microbial grazers, plants and earthworms. Plants may be benefitted from the microbial grazing through increased nutrient availability (microbial loop) and grazing induced release of root growth hormones (non nutritional effects) (Bonkowski et al., 2000a).

1.3 The roles of nematodes in nutrient cycling and plant growth

Nematodes play significant roles in nutrient cycling because of their diversity, ubiquity, and key positions in the soil food web. They acquire energy and nutrients from a range of organisms such as microbes, plants, and other fauna through microbial grazing, piercing plant roots and predation respectively. Such feeding behaviour makes them interact with several organisms that regulate the process of nutrient mineralization and cycling. The role of nematodes in nutrient cycling has long been recognized (Banage, 1963; Coleman et al., 1978a). Several studies have been conducted to understand the extent and mechanism of nematodes involvement in nutrient cycling and plant growth. These studies have reported the contribution of nematodes to the mineralization and cycling of nutrients, particularly carbon, nitrogen and phosphorus.
1.3.1 Quantifying the contribution of nematodes to nutrient mineralization

Efforts to quantify the contribution of nematodes to C mineralization (respiration) probably started when Nielsen (1949) determined respiration rates for a few selected species of nematodes in a laboratory study. Since then, several studies assessed the energy budget parameters and energy conversion efficiencies of nematodes in an effort to measure the activity of the nematode populations in different ecosystems (Ferris, 2010b; Sohlenius, 1980). Based on these parameters, the relative importance of nematodes in soil respiration has been determined.

Studies that calculated respiration by nematodes indirectly from oxygen consumption rates and estimated biomass of individual nematodes reported a small contribution of nematodes to the total soil respiration. Data from different forest ecosystems indicated that nematodes were responsible for between 0.8-2% of the total heterotrophic soil respiration (Kitazawa, 1977; Reichle, 1977; Sohlenius, 1980). Related studies estimated that nematodes may contribute between 10 to 15% of the soil animal respiration (Sohlenius, 1980). Data on the contribution of nematodes to C mineralization based on direct experimental measurements of CO₂ are not only rarely available, but also primarily done for a few selected species of bacterivorous nematodes only. However, these studies showed a significant contribution of nematodes to C mineralization. For instance, Coleman (1977) found that the presence of a bacterivorous nematode increased cumulative CO₂-C mineralized by 50% and 27% over the presence of bacteria only in glucose amended and unamended treatments, respectively. In another experiment in sterilized and glucose amended soil microcosms, Coleman et al., (1978a) found that nematodes respire 30 times more CO₂ per unit biomass than amoebae. Other experimental measurements also found that nematodes may respire between 30-85% of the assimilated C showing their possible significant role in C cycling in the soil as well (Ferris et al., 2012; Marchant and Nicholas, 1974).

The contribution of nematodes to N mineralization has received much attention from soil ecologists in the past decades. Early works by Nielsen (1949) showed an increase in N mobilization in the presence of nematodes. During the end of the 1980s and beginning of 1990s, several studies came up with experimental evidences that bacterivorous and
fungivorous nematodes significantly increased N and P mineralization. Coleman et al. (1978) reported that nematodes remineralized 50% more N after 17 days of incubation and increased net inorganic P by 43% over bacteria alone treatments. Anderson et al. (1983) found significantly more NH$_4^+$-N and Pi in microcosms containing two different species of bacterivorous nematodes than in bacteria only microcosms after ten days of incubation. In a long term microcosm experiment, Bouwman et al. (1994) found that the presence of nematodes increased C and N mineralization only until the first and second month, respectively, and reduced mineralization during the rest of incubation period. Further experimental measurements showed that fungivorous nematodes also significantly increased N mineralization in microcosms with sterilized sand amended with different organic materials with a range of C:N ratios (Chen and Ferris, 1999, 2000). Hunt et al. (1987) using a food web model of short grass prairie, calculated that 37% of the total mineralization was due to the contribution of fauna, of which 22% was by nematodes. They also reported bacterivorous nematodes and protozoa to be responsible for 83% of N mineralized by the total fauna. Under field conditions, bacterivorous and predatory nematodes were estimated to contribute (directly and indirectly) about 8% and 19% of nitrogen mineralization in conventional and integrated farming systems, respectively (Beare, 1997; Neher and Powers, 2004).

On the other hand, Bardgett and Chan (1999) found no increased N and P availabilities in microcosms inoculated with nematode community dominated by bacterivores as compared to sterilized microcosms. Buchan et al. (2013) recently found no convincing evidence for a significant direct contribution of the entire free-living nematode community to total nitrogen mineralization in disturbed and undisturbed soil cores. However, they reported 87% and 23% more NO$_3$-N in the presence of nematodes in undisturbed and disturbed cores, respectively, by the end of the three months incubation experiment. Xiao et al. (2010) found that the presence of bacterivorous nematodes significantly increased nitrification in sterilized and reinoculated soil cores, indicating further roles of nematodes in regulating N transformation.

1.3.2 Contributions of nematodes to plant nutrient uptake and growth

The roles of nematodes in stimulating plant growth may be directly through increased plant nutrient uptake (nutritional effect) and/or indirectly through modification of microbial derived
plant growth hormones (non nutritional effect) (Alphei et al., 1996; Ingham et al., 1985). Relatively few studies have been conducted to understand and quantify the contribution of nematodes to plant nutrient uptake and plant growth. These studies have indeed revealed that nematodes not only contribute to organic matter decomposition, but also to plant nutrient uptake and growth directly and indirectly. The pioneer work of Ingham et al. (1985) in this regard showed that bacterivorous nematodes increase initial plant growth and N uptake as compared to plants in soil with only bacteria. They attributed the changes to an increased N mineralization by bacteria, NH$_4^+$-N excretion by nematodes, and greater initial exploitation of soil by plant roots. In the same experiment fungivorous nematodes did not increase N uptake but significantly increased P uptake throughout the 105 days of incubation.

Recently, Irshad et al. (2011) studied the role of a bacterial feeding nematode in N and P uptake in *Pinus pinaster* seedlings planted in sterilized nutrient solution. The presence of nematodes resulted in a significant increase in plant N and plant P uptake over sterilized control seedlings and seedlings reinoculated with bacteria only. Djigal et al. (2004) also found that the presence of a bacterivorous nematode increased N uptake by 17% and plant growth by 12%. In contrast to these reports, Bardgett and Chan (1999) found no positive effects of bacterivorous dominated nematode community on N and P mineralization and uptake in Montane grass land ecosystems. Griffiths et al. (1999) also reported no enhanced growth of rye grass seedlings in the presence of two species of bacterivorous nematodes as compared to control (bacteria only) treatments.

The roles of some important root parasitic nematodes in plant nutrient uptake and growth has also been investigated despite the known yield losses they caused. For instance, Tu et al. (2003) determined the direct contribution of a root parasitic nematode (*Rotylenchulus reniformis*) to N mineralization in the presence of cotton plants and found 17–117% increased N mineralization in the presence of nematodes over treatments without nematode. A series of experiments have also examined the influence of obligate nematode herbivores of white clover (*Trifolium repens* L.), in grassland ecosystems (Bardgett et al., 1999a; Yeates et al., 1999a). In contrast to the expected plant biomass loss, Bardgett et al., (1999a) found that grazing of white clover by nematodes even increased the root biomass of the perennial rye grass which was grown alongside the white clover by 217%.
Indirect (non nutritional) roles of nematodes in stimulating plant growth have also been recorded in literature. Such roles include change in root morphology and stimulation of root growth hormone production such as IAA (Haase et al., 2007; Mao et al., 2007). These roles are often related to the associated changes in microbial community structure as a result of plant infestation (Bardgett et al., 1999a).

1.4 Mechanisms of nematode involvement in nutrient cycling and plant growth stimulation

In each of the studies conducted to quantify the role in and contribution of nematodes to nutrient mineralization and plant growth, explanations have been given in an effort to answer a related question: what mechanisms are responsible for such important roles. Several authors have proposed direct and indirect mechanisms that indicated how nematodes contribute to and regulate nutrient cycling.

1.4.1 Direct mechanism

The direct mechanism of nematode involvement in nutrient mineralization refers to the direct release of nutrients in mineral or readily mineralizable forms such as NH$_4^+$, PO$_4^{3-}$, urea and amino acids by nematodes which is in excess of their metabolic need (Ferris et al., 2012) (Figure 1.3). This mechanism mainly occur as a result of their relatively low respiration efficiencies and differences in C:N ratios between nematodes and their prey. It has been estimated that nematodes respire between 40-85% of the assimilated C (Ferris et al., 2012; Marchant and Nicholas, 1974; Sohlenius, 1980). During catabolism, then, the end products that are in excess of their metabolic need must be removed from their body. In fact, like other animals, nematodes also cannot store nutrients such as nitrogen in appreciable amounts (Wright, 2004; Wright, 1995). Depending on other environmental factors such as water availability, they may excrete excess nutrients in mineral or readily mineralizable forms. In moist conditions where water can be freely exchanged with the environment, the most energetically efficient nitrogenous excretory product for nematodes is ammonia. However, where water exchange is more limited, ammonia is generally converted in their body to either urea or a purine prior to excretion. It has been reported that amino acids and peptides
accounted for as much as 49% of the total N excreted by free-living and parasitic nematodes (Anderson et al., 1983).

It is generally accepted that nematodes have higher C:N and C:P ratios than their prey. Anderson reported C:N ratio of plant parasitic nematodes ranging between 7.5:1 to 12:1 based on measured N contents of nematodes and an assumed 50% C content of nematodes. Mckercher et al. (1979) reported 0.45% P content of dorylaimid nematodes. The C:N ratios of bacteria have often been reported to be around 5:1 (Hunt et al., 1987). Bacterivory could be the major mode of feeding among nematodes, as omnivorous and predatory nematodes are also assumed to feed mainly on bacteria. Between 75% and 90% of the total fauna has been reported to feed on bacteria (Bouwman and Zwart, 1994; Yeates, 1976). These reports indicate that a significant amount of nutrients, particularly N, is mineralized because of bacterial grazing. Taking into consideration that OM (substrate for bacteria) generally has a C:N ratio of over 10, it has even been suggested that bacterivores may mineralize relatively more N than bacteria. The difference in C:N between fungivorous nematodes and their primary prey (fungi) is small (Chen and Ferris, 2000; Ingham et al., 1985). Chen and Ferris (1999) experimentally measured C:N ratios of the body contents of two fungal-feeding nematode species of Aphelenchus and found ratios of between 8.1:1 and 10.9:1. They also found C:N ratios of 8:1 to 8.9:1 for two species of saprophytic fungi used in the same experiment as a source of food for the fungivorous nematodes. Thus, in such case not the difference in C:N ratio, but rather the low respiration efficiencies of nematodes may be the mechanism for significant increase in N mineralization.
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1.4.2  Indirect mechanisms

In addition to the direct release of nutrients to the available nutrients pool, nematodes indirectly contribute to nutrient mineralization by increasing the labile OM pool and stimulating microbial activities in several ways, such as stimulating root exudates production, defecation and phoretic activities. Herbivorous nematodes mainly contribute to decomposition indirectly, presumably due to their plant parasitic activities and their relatively low assimilation efficiency (0.26) (Hunt et al., 1987). Such interactions between plants and herbivorous nematodes result in the release of relatively high amounts of readily

Figure 1.3. A scheme for possible mechanisms how nematodes are involved in nutrient cycling. The herbivores and fungivores increase DOM pool in the form root exudates from plants and defecation. Microbial grazers, primarily bacterivores increase available nutrient pool directly by excreting excess nutrients. The activity of microbes is stimulated by each feeding group of nematodes which in turn stimulates mineralization from both pools. Nutrient uptake may further be enhanced through the release of root growth hormones by these stimulated microbes.
mineralizable organic matter that stimulates microbial biomass and activity and the subsequent indigenous OM decomposition. This increased microbial biomass in turn stimulate microbial grazers (bacterivorous and fungivorous nematodes) which directly release nutrients to the soil through the microbial pool mechanism as explained earlier. Studies suggested that the indirect contribution of plant-feeding nematodes to N mineralisation may be much higher than their direct contribution which was 2-5% of total N mineralization (Verschoor, 2002).

Microbes may further be stimulated as they are ingested and stick on the nematode cuticle and thus are transported in the soil from resource poor to resource rich microsites (phoretic activity). It has been reported that more than 30% to 90% of the bacteria ingested by nematodes are defecated alive (Freckman, 1988), giving them further opportunity to continue in OM decomposition. Nematodes may also facilitate the degradation of organic matter in the soil indirectly by releasing enzymes such as amylase and cellulase (Myers, 1966) and by improving oxygen availability in the very vicinity of microbes as they move in the soil solution (Baath et al., 1981).

1.5 Approaches to quantify the contribution of nematodes to nutrient cycling

The contribution of soil organisms to OM decomposition and nutrient cycling has been determined both through experimental approaches and through soil food web modelling. The food web models categorize the soil biota into functional groups, mainly based on feeding relationships and other life history traits such as assimilation efficiency, reproduction, and death rates. These models use such physiological parameters and the differences in C:N ratios of predators and the corresponding prey in a particular soil food web to estimate the contributions of different functional groups to net N and C mineralization (de Ruiter et al., 1994; de Ruiter et al., 1993; Hunt et al., 1987; Moore et al., 2003). According to these models, the roles of protozoa and bacterivorous nematodes have been significantly important in the N dynamics of the soil (Hunt et al., 1987).

Despite the wealth of useful information that has been acquired using these theoretical food web models, some important limitations remain unsolved and thus the outputs need careful interpretation. The main limitation is that the input parameters such as the size and turnover
rates of the population, C:N ratio of the predator and prey, assimilation and production efficiencies are difficult to experimentally measure and hence have to be often estimated. This means that the estimated contribution of a given functional group will change if the C:N ratio of one of the prey is to be modified. In his review Brussaard (1998) noted that in food web models, the soil OM is categorized into two main pools (roots and detritus) without further division into different functional pools, unlike the mechanistic SOM models. Moreover, abiotic factors predominantly influence the C and N dynamics in a given system, which food web models do not take into account. Despite these limitations, the outputs of food models were found to be comparable with experimentally measured data, N budget analysis and estimated values using other simulation models (de Ruiter et al., 1993).

According to Hunt et al. (1987) the experimental approaches to study the contribution of a group of organism to organic matter decomposition and nutrient cycling can be summarized into three categories: 1) additive methods 2) exclusion methods 3) whole system approaches. In the first method (Addition method), the soil samples become free of any living organism through different sterilizing techniques such as autoclaving, microwaving and gamma irradiation. Then, depending on the objective of the experiment, food webs are reconstructed ranging from a very simple trophic assemblage with two species to more complex food webs. This method has been widely used in microcosms for soil ecology studies (Anderson et al., 1983; Chen and Ferris, 1999; Coleman et al., 1978a; Ingham et al., 1985; Xiao et al., 2010). The limitation with this approach is that it is very simplified and that it is difficult to extrapolate the findings to field conditions (Hunt et al., 1987).

The second approach uses selective exclusion of the target organism with minimal disturbance of the system by applying techniques such as biocides, freezing, gamma irradiation and microwaving. Such techniques have been applied in making mesocosms for field studies. The limitations in these methods lay in the existence of non-target effects. For instance, Ingham and Coleman (1984) found that biocides commonly affect non-target organisms and it is not exactly known whether the observed effects in mineralization result because of the selective removal of the target group or represent secondary effects on non-target organisms.
The third approach consists of periodically determining the population dynamics of relevant functional groups of organisms under field conditions. In this approach, the functional role of a given organism is derived from correlation analysis with the nutrient concentrations (Wang et al., 2004a; Wang et al., 2004b). The limitation of this approach is that it does not measure direct contribution, but rather depends on correlation analysis which may not always show the true cause-effect relationships.

1.6 Sterilizing /defaunating tools for studying the roles of nematodes in nutrient cycling

The contribution of nematodes to nutrient cycling has often been quantified by simple differences in nutrient content between sterilized/defaunated samples reinoculated with nematodes and without nematodes. The assumption is that both samples have the same biochemical properties except the presence or absence of nematodes to which any difference in biochemical properties is attributed. However, sterilized samples usually show significant differences in biological, chemical and physical properties as compared to untreated fresh soil. Realistic defaunating methods for the investigation of nematode contribution to nutrient cycling should entirely eliminate nematodes while leaving other biological and chemical properties, particularly microbial community and nutrient concentration, intact. Because the direct and indirect contribution of nematodes to nutrient cycling is through microbial grazing, the microbial community should remain as intact as possible both in terms of biomass and community structure. Different techniques ranging from complete sterilization by autoclaving to defaunation by freezing have been applied in previous studies. The advantages and limitations of the commonly applied tools in such studies are discussed below with particular emphasis on their application in quantifying the roles of nematodes in nutrient cycling.

1.6.1 Autoclaving

Autoclaving is one of the most commonly used sterilizing technique in determining the contribution of nematodes to nutrient cycling in various media namely solid agar media (Ferris et al., 1995; Irshad et al., 2011), sand (Ferris et al., 1998); and soil (Coleman et al., 1978a; Ingham et al., 1985; Xiao et al., 2010). The duration and frequency of autoclavage varied from twice to three times within 24 hours (Ferris et al., 1998; Ingham et al., 1985). The method
is known to create nutrient flushes to strongly alter soil structures (Lotrario et al., 1995) and to inhibit more strongly the proliferation of inoculated microorganisms and plants compared to other techniques such as irradiation (Salonius et al., 1967). Given that autoclaving completely eradicates living organisms, reinoculation of the organisms including microbes is mandatory, but reconstructing the representative microflora through reinoculation is extremely difficult to achieve. Moreover, the reinoculated microbes may prefer to initially occupy the easily accessible pore spaces and thus be more exposed to microbial grazers, resulting in overgrazing and possible overestimation of the contribution of microbial grazers to nutrient cycling (Xiao et al., 2010). Given that the major contribution of nematodes to nutrient mineralization is through microbial grazing, the reported role of nematodes based on autoclaving may possibly be an overestimation.

1.6.2 Nematicides

Nematicides such as Carbofuran have been used to selectively kill nematodes in ecological studies (Chelinho et al., 2011; Ingham, 1985; Wright and Coleman, 1988). In all cases, Carbofuran significantly reduced the total abundance and the abundance of particular trophic groups, but did not entirely eliminate nematodes. This is because nematicides usually have no direct lethal effects on target nematodes, but limit their mobility and thus the ability to infect the plant hosts as is the case for carbofuran (Chelinho et al., 2011; Wright and Womack, 1981). Nematicides have non target effects notably on some earthworm taxa. More important is that its residual effect and the C flush from dead biomass and N incorporated from the chemical itself likely results in experimental errors (Wright and Coleman, 1988).

1.6.3 Fumigation

Several gases and liquids such as ethylene oxide, propylene oxide, chloroform and methyl bromide have been used for selective (defaunation) and complete sterilization in soil ecology studies (Alphe et al., 1996; Alphe and Scheu, 1993; Bonkowski et al., 2000b; Bonkowski and Schaefer, 1997). Coleman D C et al. (1977) used propylene oxide to completely sterilize soils and study the role of nematodes and protozoa in nutrient mineralization. Methyl bromide (CH₃Br) has been used to defaunate a bulk soil that was used to make an artificially reconstructed complex food web with micro, meso and macrofauna (Davidson and Grieve,
2006). It has been reported that ethylene and propylene oxide can increase the soil pH during fumigation due to esterification of carboxyl groups in soil organic matter (Trevors, 1996). Griffiths (1990a) found that the growth of a bacterial, protozoan and nematode species was inhibited in soil sterilized with ethylene oxide, most likely due to residues of ethylene chlorohydrin and ethylene glycol from the sterilization process. Chloroform fumigation was also used to defaunate the soil in studies aimed to quantify the contribution of nematodes to N mineralization. A single chloroform fumigation and incubation did not kill all nematodes initially (Alphei and Scheu, 1993), but later the application of two chloroform fumigation incubation cycles was tested and found to completely kill nematodes and protozoa (Alphei et al., 1996; Griffiths et al., 1999). However, fumigation also kills the microflora (Alphei et al., 1996).

1.6.4 Freezing and thawing

Freezing and thawing is one of the most commonly used defaunating techniques in soil ecological studies. It appeared to be effective in eliminating nematodes and other fauna from the soil in several ecological studies (Bardgett et al., 1998; Djigal et al., 2004; Viketoft, 2008). There are some differences among studies that used freezing and thawing techniques for defaunation, particularly in terms of the temperature, frequency and duration of freezing thawing cycles. Bardgett et al. (1998) subjected sieved soil to drying at 80°C for 24h followed by freezing at -80°C for 24h. This was repeated and no nematodes were found afterwards. Kaneda and Kaneko (2007) defaunated soil samples by freezing for 24 hrs at −20°C to study interactions between nematodes and Collembola. However, they did not report whether nematodes were ultimately killed or not. Larsen et al. (2007) reported that three cycles of freezing (72 h at −18°C) and thawing (72 h at 22°C) enabled the microflora, protozoa, and nematodes to recover after defaunation, while mesofauna and earthworms were killed. Similarly, Milcu et al. (2006) applied freezing at −22°C for 14 days to selectively kill meso- and macrofauna, assuming that nematodes and protozoa remain largely intact. Djigal et al. (2004) in an effort to study the interaction of nematodes with microbes and plants adapted both the freezing thawing temperature and cycle. They found that nematodes were completely killed after subjecting the soil (rewetted to its field capacity) to five freezing and thawing cycles (7 days at -21°C and seven days at room temperature).
In addition to the variation in temperature and frequency, the method has also resulted in several non-targeted physical and biological changes. The physical changes include a decrease in aggregate stability (particularly at higher moisture contents at the time of freezing, (Oztas and Fayetonbay, 2003), and a decrease in water holding capacity (Huhta et al., 1989). More important changes, particularly in microcosm experiments, are the biological changes such as an increase in microbial respiration following the decomposition of ruptured or dead microbial cells (Deluca et al., 1992) and the subsequent nutrient flush.

1.6.5 Gamma irradiation

Gamma irradiation is a method that has been used in soil ecology at least since the 1960s in an effort to obtain sterile soil with physical, chemical, and enzymatic properties as close as possible to normal field soil (McLaren, 1969). Efforts have been made to adapt the doses and it is emerging as a preferable tool over other methods such as autoclaving and freezing because of two main reasons (McNamara et al., 2003). Firstly, it gives possibility to vary the doses in order to selectively kill a target organism, and secondly it has a smaller effect on soil physical properties. Lotrario et al. (1995) reported (after wet sieving) that lower doses, e.g. up to 10 kGy, had minimal effect on the size distribution of soil particles, while autoclaving appeared to increase the sand sized and decrease the clay sized fractions, probably by causing increased aggregation of soil particles.

Several hypotheses have been proposed and tested to explain the underlying mechanism for the lethal effect of gamma irradiation, such as the production of toxic substances in the cell, cellular membrane and cytoplasm damage, and DNA damage (Grezz et al., 1983). It appears that DNA damage is a widely accepted lethal effect of gamma irradiation (Aquino, 2012; McNamara et al., 2003; Romanovskaya et al., 1999). This lethal effect of gamma irradiation is direct and indirect. The direct effect is due to ionization, that is, the high energy directly breaks the DNA strands. The indirect effect is due to radiolysis of water, that is, the water inside and surrounding the cell dissociates into free oxygen, hydrogen and hydroxide radicals, which can damage the DNA (McNamara et al., 2003). It has been estimated that the OH radicals formed in the hydration layer around DNA molecules are responsible for about 90% of DNA damage (Aquino, 2012).
This means that the larger the soil moisture content at the time of irradiation, the larger the lethal effect of a particular dose and the more intense the breakdown of the organic matter. Higher moisture content at the time of irradiation with lower doses was found to increase initial $\text{NH}_4^+$ concentration (Lensì et al., 1991; McNamara et al., 2003; Salonius et al., 1967), betaglucosidase activity (Lensì et al., 1991), soluble carbohydrate and amino compounds (Salonius et al., 1967), soil pH (Lotrario et al., 1995) and to decrease potential $\text{CO}_2$ production (Lensì et al., 1991) and microbial counts (Jackson et al., 1967) in contrast to dry soils. High doses such as 25 or 30 kGy have been shown to kill both microbes and fauna in the soil (Lensì et al., 1991). Lower doses (<10 kGy) do not eliminate microbes, but it is possible that lower doses would kill more microbes if the moisture content at the time of irradiation would increase.

Nematodes respond differently to different soil moisture contents at a given dose of irradiation. For example, Thompson (1990) reported 5 kGy was not sufficient to completely eliminate nematodes in air dried soil, while the same dose was sufficient to completely kill nematode in fresh soils (Buchan et al., 2012). Furthermore, Popenoe and Eno (1962) showed that 2.5 kGy was sufficient to eliminate all free-living nematodes within 14 days after irradiation in sandy soil whose moisture content was reduced to 10% gravimetric moisture content. These studies suggest the importance of the moisture content of the soil at the time of irradiation. However, several studies did not consider the moisture content of the soil at the time of irradiation. For example, Buchan et al. (2012) recently recommended 5 kGy to eliminate nematodes completely without significantly changing the microflora, but the exact moisture content at the time of irradiation was not mentioned. In a series of experiments Buchan and coauthors showed the total PLFA and the biomarker PLFAs for microbial groups did not significantly change after application of 5 kGy dose (Buchan, 2013; Buchan et al., 2013; Buchan et al., 2012). However, the microbial biomass C was found to be significantly lower in gamma irradiated soil.

Despite the advantages, gamma irradiation reportedly caused nutrient flushes during and after irradiation (McNamara et al., 2003). The nutrient flush during irradiation could be mainly due to degradation of organic matter and clay (McLaren, 1969; Popenoe and Eno, 1962). McLaren (1969) reported that as the soil contains more total N and P, more extractable N and
P was formed during irradiation suggesting a close relationship between the amount of nutrients released and the soil type. However, the nutrients released from OM degradation are very small as compared to that from the killing and lysis of organisms.

The killing of organisms combined with sustained enzyme activities are responsible for the release of nutrients after gamma irradiation. As the cells die and lyse, the readily soluble organic compounds are released into the soil. The unaffected microbes (particularly if lower doses are applied) would use these substrates for their metabolic activity eventually resulting in increased respiration and release of mineral nutrients (N and P). Several extracellular enzymes such as urease, beta glucosidase, phosphatase and proteolytic enzymes remained unaffected up to 20 kGy gamma irradiation (Shih and Souza, 1978). The increase in mineral nutrient concentrations in the absence of microorganisms (following complete sterilization) suggests that mineralization may continue because of the activity of these enzymes.

1.7 Problem statement

Organic matter decomposition and nutrient cycling are crucial terrestrial ecosystem functions that involve multitrophic interactions among soil organisms such as bacteria, fungi, protozoa, nematodes, other fauna and plants. Among the soil fauna, nematodes and protozoa have been estimated to take the largest share of the faunal contribution to these functions. Particularly nematodes play a significant role in regulating nutrient cycling as they are the most abundant multicellular soil fauna with diversified feeding mechanisms. They live in the soil as plant-feeders, microbial grazers, predators or omnivores and occupy key positions in the soil food web that enable them to interact with microbes, plants and other fauna. Several studies have demonstrated a significant contribution of nematodes to soil organic matter decomposition and nutrient cycling. However, these studies are often based on theoretical food web model calculations or on simplified experimental set ups using a few selected species in inert or soil media and often without plants. Despite the great contribution of these findings to the advancement of our understanding of the roles of nematodes in soil processes and functions, they fail to take important multitrophic interactions into consideration, particularly the interactions between plants and nematodes. Consequently, these findings may be less accurate and hamper the extrapolation of the results to actual field conditions.
In order to investigate the contributions of nematodes to nutrient cycling under realistic conditions, there is a need for selective elimination of nematodes which leaves other biological and chemical properties (particularly microbial community and nutrient concentrations) intact. Recently gamma irradiation has been optimized to eliminate nematodes selectively while leaving the microbial biomass largely intact (Buchan et al., 2012). However, the nutrient flush remains high in irradiated samples. Moreover, little is known about the effect of soil moisture content at the time of irradiation on the lethal effect of a given dose and potential effects on other biological parameters such as enzymatic activities. This thesis further optimizes the gamma irradiation technique and uses it in quantifying the contribution of the entire free-living nematode community to nutrient cycling in experimental set ups that allow multitrophic interactions between native microbes, nematodes and plants.

1.8 Objectives and thesis outlines

The overall objective of this thesis is to quantify the contribution of the entire free-living nematode community to organic matter decomposition and plant nutrient uptake at different levels of below ground biotic and abiotic interactions. The central hypothesis tested throughout the thesis is formulated as follows: *interactions between native microflora, the entire free-living nematode community and plants enhance indigenous or added organic matter decomposition, nutrient cycling and plant nutrient uptake.* To test this central hypothesis, a series of microcosm experiments were conducted each aiming to answer a particular research question related to the main hypothesis. The research questions and the thesis outline are summarized as follows:

1. *What is the optimum gamma irradiation dose that eliminates nematodes without significantly altering the biochemical properties of the soil?*

Several other specific questions were included under this main question such as, 1) what is the effect of soil moisture content at the time of irradiation on the lethal effect of a given dose? 2) What is the response of soil enzymatic activities to gamma irradiation? 3) Does leaching with water after gamma irradiation reduces the nutrient flush to a level comparable to the untreated fresh soil? Chapter 2 presents the setup and findings of a microcosm incubation experiment.
2. **What is the contribution of the entire free-living nematode community to C mineralization?**

The collective contribution of nematodes to C mineralization was determined both from indigenous SOM and externally added organic matter (fresh grass-clover) during a six months incubation experiment. In this case, the optimized gamma irradiation dose was applied to sterilize the soil selectively. Chapter 3 reports the findings of this incubation experiment aimed at quantifying their collective effects on C mineralization and testing whether their contribution significantly differs in indigenous OM and added fresh OM.

3. **How do interactions between the entire free-living nematode community, native microflora and plants influence N mineralization from indigenous OM and plant N uptake?**

Here, the evolution of N mineralization, and microbial properties such as microbial biomass C and selected enzymes were monitored in bare and planted microcosms during a three months incubation. Chapter 4 discusses these findings attempting to show how the plant interactions affect the role of microbes and nematodes on nutrient dynamics by comparing with their effect in bare soil.

4. **How do interactions between the entire free-living nematodes, indigenous microflora and plants influence nitrogen, phosphorus and sulfur mineralization and plant nutrients uptake in grass-clover amended microcosms?**

This last incubation experiment was conducted to quantify the collective contribution of nematodes to major nutrients (N, P and S) cycling and plant uptake during the decomposition process of indigenous and added fresh OM in microcosms planted with Italian rye grass. In Chapter 5, the findings of this investigation are discussed by comparing with the previous experiment (Chapter 4) with a similar setup but without amendment.

5. **What is the collective role of free-living soil nematode community in native or exogenous OM decomposition and nutrient cycling in the absence and presence of plant interactions?**

Chapter 6 presents the main findings of the thesis the extent and the mechanism how the entire free-living nematode community influences microbial properties, nutrient cycling and plant nutrient uptake at different levels of interactions.
Chapter 2:

Adapting Gamma irradiation as a tool for quantifying the contributions of nematodes to nutrient cycling
Abstract

Gamma irradiation is a tool in soil ecological studies to selectively kill target organisms, based on the dose used. Changes in soil biochemical properties are also less as compared to autoclaving and freezing. Although gamma irradiation has often been used in studying the roles of nematodes in nutrient cycling, the recommended doses to eliminate nematodes still lead to a nutrient flush and, moreover, are not 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 under different soil moisture contents. 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 contents (air dried, 50% 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 the nutrients from the flush. The results showed that at 80% WFPS, nematode abundance, enzyme activities and total mineral N concentrations were lower than at 50% WFPS during most of the incubation period at each irradiation dose. A higher moisture content, however, did not make significant changes in total phospholipid fatty acid concentration at any level of irradiation, except at 3 kGy. These findings indicate that the effects of gamma irradiation on soil biological and chemical properties vary depending on the moisture content of the soil at the time of irradiation. Hence, the moisture content of the soil at the time of gamma irradiation needs to be considered when using gamma irradiation as a tool in selective sterilization or defaunation. Leaching the 50% WFPS samples irradiated at 5 kGy dose immediately after irradiation significantly reduced both NH$_4^+$-N and NO$_3^-$-N concentrations, but NH$_4^+$-N concentration remained higher compared to the unirradiated control. This indicates that leaching with water only partly reduced the nutrients from the flush and underlines the need to test additional methods to remove the nutrient from the flush further. The application of 3 kGy at 80% WFPS at the time of irradiation was found to be the most effective in eradicating nematodes and leaving comparable microbial abundance and community structure as in the unirradiated control.
2.1 Introduction

The contributions of nematodes to nutrient cycling have often been 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). This type of experimental setup has the assumption that both intact and sterilized samples have the same properties except the presence or absence of nematodes to which then any observed differences in biochemical properties are attributed. The drawback to this straightforward approach, however, is that sterilized samples, usually, exhibit significant differences in biological, chemical and physical properties as compared to untreated fresh soil (Alphei and Scheu, 1993; Powlson and Jenkinson, 1976). Findings based on such disturbed soils, thus, may not be representative and not easily extrapolated to the field conditions.

Realistic defaunating methods for the investigation of the contribution of nematodes to nutrient cycling should eliminate nematodes without causing significant alterations to other biological and chemical properties, particularly microbial community and nutrient concentration. At present, however, there is no sterilizing and / or defaunating technique that does not impact the microbial biomass and nutrient concentrations. For example, the most widely used method in soil ecology studies which is autoclaving, 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, 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 direct and indirect contribution of nematodes to nutrient cycling is mainly through microbial grazing. Therefore, changes in microbial biomass and community structure may eventually lead to an unrealistic estimation of their contributions. The other method often used for soil defaunation is freezing and thawing, which appears to be effective in defaunating soil samples (Djigal et al., 2004). However, it significantly changes the physical properties and more importantly biological properties such
as an increase in microbial respiration following the decomposition of ruptured or dead microbial cells (Deluca et al., 1992).

Another technique that has been used in soil ecology studies and recently in nematode studies, is gamma irradiation. It is emerging as 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 (Lensin 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). An application of 5 kGy dose has been recommended recently and used to study the contribution of nematodes to nitrogen mineralization in sandy loam soil (Buchan et al., 2013; Buchan et al., 2012; Gebremikael et al., 2014b).

Despite the advantages of using gamma irradiation, a number of concerns such as nutrient flushes during and after irradiation and the reproducibility of the method remain unsolved. 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 concentrations resulting in overestimation of their contribution compared to the control. Initial high nutrient concentrations following soil sterilization or defaunation in microcosms can also influence the net availability of nutrients, particularly N through effects on immobilization.

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 into free oxygen, hydrogen and hydroxide radicals potentially damaging the DNA (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. For example, Thompson (1990) reported that 5 kGy was not sufficient to completely eliminate nematodes in air dried soil while the same dose was sufficient to completely kill nematodes in fresh soils (Buchan et al., 2012). Furthermore, Popenoe and Eno (1962) showed that 2.5 kGy was sufficient to eliminate all free-living nematodes within 14 days after irradiation in sandy soil with a gravimetric moisture content of 10%. Previous studies assessed the effects of moisture content of the soil on the efficiency of a given dose of gamma irradiation often with a limited number of moisture contents (dry and wet) and looking at effects on bacterial populations (Jackson et al., 1967). To the best of our knowledge the effect of soil moisture content on irradiation efficiency has never been tested on soil fauna, particularly on nematodes. To reduce the high nutrient concentration after sterilization, leaching with water is often used, however, no data is available to what extent this technique has reduced nutrient concentration over time (Alphei et al., 1996; Bonkowski and Clarholm, 2012; Koller et al., 2013a).

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) lysis of killed cells may result in the flush of more dissolved organic compounds that eventually lead to an increased nutrient concentration. Reducing the dissolved organic compounds by leaching with water after gamma irradiation may decrease the nutrients in the flush after irradiation. To test these hypotheses, we applied a range of low gamma irradiation doses on soil samples at three moisture contents and did an incubation experiment for about 3 months. The effects of each dose-moisture combination on microbial biomass and community structure, enzymatic activities, nitrogen mineralization and nematode abundances were determined and compared. Moreover, we studied whether leaching with water immediately after gamma irradiation could reduce the C and N flush.

2.2 Materials and Methods

2.2.1 Sample collection, preparation and gamma irradiation

Composite soil samples were collected from the 0-15cm layer of an organically managed agricultural field at Merelbeke, Belgium, sown with grass-clover mix. The soil texture was sandy loam with a bulk density of 1.49 g cm\(^{-3}\). At the time of sampling, the soil was
characterized by 1.21% organic C, 0.11% total N and 43.8 mg mineral N kg\(^{-1}\) dry soil. The composite sample was gently sieved through a 5 mm mesh in order to homogenize it and remove stones and visible soil animals such as earthworms. Part of this homogenized soil was spread at room temperature for air drying while the remaining soil was kept in a refrigerator (4°C). After a few days, the samples were removed from the refrigerator and filled into large sized PVC columns (height = 30 cm and diameter =10.8 cm) at a bulk density according to the field measurement. The moisture content of each sample in the PVC columns was adjusted either to 80% water filled pore space (WFPS) or to 50% WFPS. The soil volumetric moisture content at sampling was calculated from the soil bulk density and the gravimetric moisture content at sampling. The respective amount of water to be added was then calculated as the difference between the volumetric moisture content of the target water-filled pore space (either 50 or 80% WFPS) and the initial volumetric moisture content. The air dried soil was also filled into PVC tubes of the same size.

Part of the PVC columns from the air dried and moist soils (50 and 80% WFPS) were left unirradiated as a control. The remaining PVC columns that were filled with either air dried or moist soil (50 and 80% WFPS), received either 1, 3 or 5 kGy doses of gamma irradiation at the Synergy Health sterilizing company, Etten-Leur, The Netherlands. The exact dose applied to each PVC column was measured by a dosimeter. The average dosimeter readings were 0.9, 2.9 and 4.5 kGy for the intended 1, 3 and 5 kGy doses respectively (diagram representation of the soil preparation procedures followed for gamma irradiation is given in Figure 3.1).

### 2.2.2 Leaching with water

To test whether leaching immediately after irradiation reduces the nutrient flush or not, we only considered the moist soil samples with a moisture content of 50% WFPS and irradiated with 1, 3 and 5 kGy dose. For this purpose, the bottom of each PVC column (same as used for irradiation) was covered with a 5µm nylon mesh that does not allow nematodes to pass through. Half of the soil with a moisture content of 50% WFPS (from the control and irradiated samples) was transferred into these PVC tubes (with nylon mesh). The soil in each tube was then saturated by adding an appropriate amount of demineralized water slowly and left for a few hours to let the water distribute homogeneously throughout the soil in the PVC tubes.
Finally, suction from -300 to -700 millibar was gradually applied and the leachate was collected through a tube that connected a glass funnel attached at the bottom of each PVC tube into a separate flask.

2.2.3 Moisture content adjustment

The incubation following the application of gamma irradiation was carried out at a moisture content of 50% WFPS, which was done as follows. The moisture content of the samples irradiated at 80% WFPS was reduced to slightly lower than 50% WFPS immediately after irradiation by spreading the soil samples at room temperature. The moisture content of the leached soil samples was also reduced to slightly lower than 50% WFPS immediately after finishing the leaching in the same way as the samples with a moisture content of 80% WFPS. The moisture content of the air dried soil samples was adjusted to 50% WFPS by adding an appropriate amount of demineralized water a day before the incubation experiment was started.

2.2.4 Treatments and experimental setup

For each gamma irradiation dose (1, 3 and 5 kGy), four treatments with different moisture content at the time of irradiation were compared to the respective unirradiated controls (CTR) and amongst each other. These were; 1) air dried (AIRDRIED), 2) moist soils with 50% WFPS (50%WFPS), 3) moist soils with 80% WFPS (80%WFPS) and 4) moist soils with 50% WFPS but leached with water immediately after irradiation (LEACHED). As mentioned above soil samples from treatment 1, 3 and 4 were also brought to 50% WFPS before the start of the incubation.

A total of 240 experimental units were prepared by filling small sized PVC cores (height=7.5cm and diameter=7.5cm) with 250 g of the respective moist soil to a bulk density of 1.4 g cm$^{-3}$. The field bulk density was not exactly reconstructed in this experiment, as it was difficult to compress the soil without making compaction. The moisture content of each sample was adjusted to 50% WFPS and kept constant throughout the incubation experiment by adding demineralized water every week, based on weight differences. The incubation experiment was carried out in a growth chamber at a constant temperature (17°C) and light (16/8 day/night) regime. Three replicates of each treatment were removed and destructively sampled at the start and after 7, 21, 42 and 84 days of incubation for nematode extraction,
chemical and biological analyses. Based on visual observation, the moisture content was uniform throughout the cores.

2.2.5 Nematode Extraction and counting

Throughout the incubation, all free-living nematodes were extracted from 70 g moist soil using an automated zonal centrifuge machine that separates free-living nematodes in water suspension from mineral and most organic soil constituents based on their specific density (Hendrickx, 1995). After extraction, the nematode suspension was poured onto a sieve covered with a filter paper for 72 hours at room temperature (±20 °C). Only viable nematodes that migrated towards the water were collected in the bottom of the plate, concentrated and counted using a dissecting microscope (40x).

2.2.6 Microbial biomass carbon and community structure

Microbial biomass carbon (C_{mic}) was determined using 30 g of fresh soil by fumigation-extraction method according to Vance et al. (1987). The extraction efficiency coefficient used for C_{mic} calculations was 0.45 (Joergensen, 1996).

Changes in the structure of the microbial community were determined based on microbial membrane phospholipid fatty acids (PLFA) following a procedure explained in details by Moeskops et al. (2010) and Buchan et al. (2012). Briefly, 4 g of freeze-dried soil from each sample was sieved (2mm) in order to homogenize and remove the root fragments and stones. This freeze-dried soil was subjected to different extraction procedures for three days. On the first day, total lipids were separated from other soil components using a multi-phase extraction mixture (CHCl_3, MeOH and P buffer) in a separatory funnel. On the second day, the extracted lipids were fractionated into three lipid classes using Solid Phase Extraction (SPE) cartridges, after which only phospholipids were kept for further analysis. The separated PLFAs were transformed into fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. After drying the FAMEs under N_2 gas, the FAMEs were re-dissolved in hexane containing nonadecanoic acid methyl ester (C19:0) as an internal standard FAME. Finally, individual FAMEs were identified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS) on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Interscience BVBA, Louvain-la-Neuve, Belgium) in electron ionization mode.
The sums of marker fatty acid concentrations for selected microbial groups were calculated as follows. For Gram-positive bacteria the sum of i15: 0, a15: 0, i16: 0, a16: 0, i17: 0 and a17: 0; for Gram-negative bacteria cy17: 0, cy19: 0, C16:1ω7, C16:1ω9; for the actinomycetes the sum of 10-methyl branched saturated fatty acids (Buyer and Sasser, 2012; Frostegard et al., 1996; Kaiser et al., 2010). For the total bacterial community the fatty acids 15:0, 17:0 and cy19:0 were also included in addition to the sum of marker PLFAs for Gram-positive bacteria, Gram-negative bacteria and actinomycetes. For saprotrophic fungi the marker PLFAs 18:2ω6c and 18:1ω9; and for arbuscular mycorrhizal fungi (AMF) 16:1ω5c were considered (Joergensen and Wichern, 2008). The bacterial: fungal ratio was calculated as the sum of total bacterial marker fatty acids divided by the saprotrophic fungal marker fatty acids.

2.2.7 Enzymatic activities

Dehydrogenase activity was determined using Triphenyltetrazolium Chloride (TTC) as a substrate following a procedure by Moeskops et al. (2010). Briefly, 2 ml of 3% TTC and 2 ml of Tris-buffer pH 7.8 was added into vials containing 5 g moist soil. The samples were incubated for 24hrs at 37°C and put on a linear shaker (125 rev min$^{-1}$) for 2hrs after adding 20 ml of methanol. The extractant was then filtered through Whatman no. 5 filter and color intensity of the filtrate was measured using a Varian Cary 50 spectrophotometer at 450 nm. The same procedure was followed for the control except that 4 ml of Tris-buffer was added instead of 2 ml TTC. Dehydrogenase activity for each sample was determined as the difference between the sample and the control.

β-glucosidase activity in the soil samples was determined using p-nitrophenyl-β-D-glucoside as a substrate according to the procedure by Alef and Nannipieri (1995). One gram moist soil was weighed in triplicate in glass vials and 4 ml of modified universal buffer and 1 ml of 25 mM p-nitrophenyl-β-D-glucoside was added, thoroughly mixed and incubated for 1hr at 37°C. After incubation, 1ml of 0.5M CaCl$_2$ and 4 ml Tris buffer pH 12 was added and immediately filtered through Whatman no. 5 filter paper. The same procedure was followed for the control samples except that the substrate was added after incubation. The color intensity of the filtrate was measured at 400 nm with a Varian Cary 50 spectrophotometer.
2.2.8 N mineralization

Nitrogen mineralization was determined by measuring the evolution of mineral nitrogen (NH$_4^+$ and NO$_3^-$) throughout the incubation. Both NH$_4^+$ and NO$_3^-$ were measured from the same aliquot colorimetrically in a continuous flow auto analyzer (Chem-lab 4, Skalar 223 Analytical, Breda, The Netherlands), following the extraction of 30 g moist soil with 60 ml 1M KCl (1:2 ratio) after shaking for one hour.

2.2.9 Statistical analysis

The experiment followed a factorial design with three fixed factors, each with four levels: Factor 1) moisture content: air dried, 50% WFPS, 80% WFPS, and 50% WFPS but leached with water, Factor 2) gamma irradiation doses: 0 kGy (non irradiated control), 1, 3 and 5 kGy, Factor 3) incubation time: 7, 21, 42 and 84 days after incubation. The data were checked for normality and homoscedasticity. Most of the variables showed a normally distributed pattern, but the variances were heterogeneous. Several transformations did not help to solve the heterogeneity problems. A weighted least square method which gives more weight to the group means with lower standard deviation was applied to homogenize variances (Kutner et al., 2005). A three way ANOVA (full factorial model) was initially applied, but resulted in a significant three way interactions for most of the variables, which makes the interpretation more complex. Alternatively, the whole file was split into four based on the factor gamma irradiation doses. Then two way ANOVA model was used separately for each gamma irradiation dose. The differences between mean pairs were tested using Bonferroni with its correction. The PLFA data were further analyzed by Principal Component Analysis (PCA) based on the correlation matrix after checking the sampling adequacy using the Kaiser-Mayer-Olkin (KMO) test. All statistical analyses were done using IBM SPSS Statistics software package version 20 (SPSS inc., Chicago, USA).

2.3 Results

2.3.1 Nematode abundances

Living nematode abundance significantly (p<0.05) decreased as the moisture content increased from 50 to 80% WFPS in all the irradiated samples and the unirradiated control
Adapting gamma irradiation throughout the incubation period (Figure 2.1). In the AIRDRIED samples, nearly no living nematodes were found both in the CTR and irradiated samples. LEACHED samples showed no significant differences in nematode abundances in the CTR and irradiated soils compared to the 50%WFPS, except on day 21 and 84 in samples irradiated with 3 kGy.

2.3.2 Phospholipid fatty acids (PLFA) profiles

At the beginning of the incubation, AIRDRIED treatments showed significantly lower total PLFA concentrations than the other treatments in the CTR samples. There were no significant differences (p>0.05) in total PLFA between moisture treatments in all levels of irradiation (Figure 2.2a). Further principal component analysis of the PLFAs showed that the first principal component (PC1) explained 49.8% of the total variation in the dataset and the second principal component (PC2) explained 27.7%. PC1 separated the unirradiated CTR and lower doses from that of all the AIRDRIED treatments and higher doses of the other treatments. PC2 clearly separated 50%WFPS and LEACHED treatments from AIRDRIED and 80%WFPS treatments regardless of the gamma irradiation doses. Biomarker PLFAs that mainly loaded PC1 were actinomycetes, gram negative bacteria, gram positive bacteria and others such as fungi and protozoa as well. Biomarkers that mainly loaded PC2, were fungi, gram positive bacteria, gram negative bacteria, actinomycetes and protozoa (Appendix 1).

At the end of the incubation, the total PLFA concentration in the AIRDRIED treatments was found to be significantly higher than in the 50%WFPS and LEACHED treatments in the CTR and at all irradiation doses (Figure 2.2b). No significant differences were observed between moisture treatments except that total PLFA was higher in 80%WFPS than in 50%WFPS at 3 kGy dose. PC1 explained 71.4% of the total variation in the dataset and PC2 explained 12.4%. Here, PC1 separated all the AIRDRIED and 80%WFPS treatments from the LEACHED and 50%WFPS treatments. PC2 mainly separated all the CTR, LEACHED and 50%WFPS from AIRDRIED and 80%WFPS treatments. Biomarker PLFAs that largely loaded PC1 were G+, G-, and other groups of bacteria, fungi and protozoa. PC2 was mainly loaded by biomarker PLFAs for actinomycetes, fungi and gram negative bacteria.
Figure 2.1. Living nematode abundance over time at different moisture content in the CTR and all irradiation levels. Unirradiated control treatment (CTR50%WFPS) is presented at each dose in order to compare with the irradiated samples. The error bars refer to standard error of the mean (n=3.).
Figure 2.2. Total PLFA concentration (nmole g⁻¹ dry soil) at the beginning (a) and end (b) of the incubation. The error bars refer to the standard error of the mean (n=3) and different small letters refer to significant differences between the treatments in the CTR and each irradiation dose.
Figure 2.3. PLFA mean scores (n=3) with standard error of the mean at the beginning (a) and end (b) of the incubation as analysed by the principal component analysis. Gamma irradiation doses are represented as C (0 kGy), 1k (1 kGy), 3k (3 kGy) and 5k (5 kGy). The moisture treatments are represented as A (AIRDRIED), 50 (50%WFPS), 80 (80%WFPS) and L (LEACHED with water). The corresponding Loadings are shown for important PLFAs that contributed to the explained variation.
The total PLFA in unirradiated 50% WFPS CTR was not significantly different compared to the 3 kGy at 80% WFPS at the beginning and end of the incubation (Figure 2.2). The 5 kGy at 80% WFPS treatments resulted in significantly lower total PLFA compared to the 50% WFPS CTR. The concentration of the biomarker PLFAs of the microbial groups tend to be generally lower in irradiated samples at the start of the incubation. Biomarker PLFAs for G+ bacteria and saprophytic fungi were not significantly different in 3 kGy at 80% WFPS compared to 50% WFPS CTR.

**Table 2.1** Comparison of the unirradiated CTR against samples irradiated with 3 and 5 kGy dose of the 80% WFPS treatments at the beginning and end of the incubation experiment. Mean differences (nmole g⁻¹) and P values from one way ANOVA output are presented.

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>time (days)</th>
<th>CTR 50% vs 3 kGy at 80% WFPS</th>
<th>CTR 50% vs 5 kGy at 80% WFPS</th>
<th>3 kGy vs 5 kGy at 80% WFPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean difference (Irradiated-CTR)</td>
<td>P values</td>
<td>mean difference (Irradiated-CTR)</td>
</tr>
<tr>
<td>G+ bacteria</td>
<td>7</td>
<td>-1.73 0.238</td>
<td>2.43 0.070</td>
<td>0.68 0.841</td>
</tr>
<tr>
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2.3.3 Enzymatic activities

Dehydrogenase activity was significantly lower in 80%WFPS than 50%WFPS during initial periods of the incubation with mean differences of 27.2, 19.9, 7.9, 7.5 µg TPF g⁻¹ day⁻¹ in the CTR, 1 kGy, 3 kGy and 5 kGy doses, respectively, on the 7th day of incubation (Figure 2.4). Similarly, significant differences were found at the end of the incubation in the CTR and all irradiated samples, although the mean differences were smaller than on the 7th day. Dehydrogenase activity rapidly increased in the AIRDRIED samples until day 42. No significant differences were observed between AIRDRIED and the other treatments at 3 and 5 kGy doses. LEACHED samples resulted in slightly lower dehydrogenase activity than 50%WFPS throughout the incubation in the unirradiated CTR samples and the samples irradiated with 1 kGy. Dehydrogenase activity was lower in the LEACHED soils than in 50% WFPS in the samples irradiated with 3 kGy and 5 kGy with statistically significant differences only on day 84 and 42, respectively.

Betaglucosidase activity was significantly higher in the 80%WFPS than 50%WFPS at the start of the incubation in the unirradiated CTR and in the samples that received 3 and 5 kGy doses with mean differences of 11.6, 15.4, 17.8 µg PNP g⁻¹ hr⁻¹ respectively on the 7th day of incubation. During the rest of the incubation period, betaglucosidase activity was lower in the 80%WFPS than 50%WFPS samples in all the samples except in the ones irradiated with 1 kGy dose. AIRDRIED samples consistently showed the lowest betaglucosidase activity in the CTR and in the irradiated samples throughout the incubation period (Figure 2.5). The mean differences between LEACHED and 50%WFPS were not consistent and no significant differences (p>0.05) were observed in all treatments except in 5 kGy on day 42.
Figure 2.4. The evolution of dehydrogenase activity over time at different moisture contents in the CTR and all irradiation levels. The error bars refer to the standard error of the mean (n=3).
Figure 2.5. The evolution of betaglucosidase activity over time at different moisture contents in the CTR and all irradiation levels. The error bars refer to the standard error of the mean (n=3).

### 2.3.4 Evolution of soil mineral N

NH$_4^+$-N concentration was significantly higher (p<0.05) in 80%WFPS than in 50%WFPS until day 21 in samples irradiated with 1, 3 and 5 kGy with mean differences of 4.1, 8.6, 4.3 µg NH$_4^+$-N g$^{-1}$ dry soil, respectively. It consistently declined in the 80%WFPS samples generally after day 21 while it consistently increased in the 50%WFPS samples with mean differences of 21.2, 27.6 and 40.6 mg NH$_4^+$-N kg$^{-1}$ dry soil in 1, 3 and 5 kGy doses, respectively, at the end
of the incubation. In the unirradiated CTR samples, no significant differences were observed between moisture treatments throughout the incubation period except at t0. AIRDRIED samples resulted in the highest NH₄⁺-N concentrations throughout the incubation period in the CTR and 3 and 5 kGy doses (Figure 2.6). LEACHED samples did not show significant differences with 50%WFPS samples in the unirradiated CTR. However, after day 42, LEACHED samples resulted in significantly lower (p<0.05) NH₄⁺-N concentration in the samples irradiated with 1, 3 and 5 kGy with mean differences 7.8, 18.0, and 25.6 µg NH₄⁺-N g⁻¹ dry soil, respectively, at the end of the incubation.

Nitrate concentration was significantly lower (p<0.05) in 80%WFPS treatments than 50%WFPS in the CTR and the irradiated samples during most of the incubation period. However, at the end of the incubation, 80%WFPS showed significantly higher (p<0.05) NO₃⁻-N concentration than 50%WFPS in the samples irradiated with 1, 3 and 5 kGy with mean differences of 6.0, 13.8 and 21.2 µg NO₃⁻-N g⁻¹ dry soil, respectively. Unlike NH₄⁺-N, AIRDRIED samples showed the lowest NO₃⁻-N concentration in the unirradiated CTR and all irradiated samples (Figure 2.7).

LEACHED samples resulted in lower NO₃⁻-N concentration than 50%WFPS throughout the incubation period in the CTR and 1 kGy. In the samples irradiated with 3 and 5 kGy doses, the same results were observed except that LEACHED samples showed no significant differences in NO₃⁻-N compared to 50%WFPS at the end of the incubation.

Total mineral N concentration in 80%WFPS was significantly lower than in 50%WFPS in the unirradiated CTR and all irradiated samples throughout the incubation period, except on day 7 and day 21 in the CTR. (Figure 2.8). LEACHED samples also consistently showed significantly lower total mineral N concentration than 50%WFPS samples in the CTR and at all irradiation doses.
Figure 2.6. The dynamics of NH₄⁺-N concentration over time at different moisture contents in the CTR and all irradiation levels. The error bars refer to the standard error of the mean (n=3).
Figure 2.7. The dynamics of NO$_3$-N concentration over time at different moisture contents in the CTR and all irradiation levels. The error bars refer to the standard error of the mean (n=3).
2.4 Discussion

The primary objective of this experiment was to investigate whether increasing the moisture content of a given soil sample at the time of gamma irradiation increases the lethal effect of a given dose on free-living nematodes. Three different moisture contents, namely AIRDRIED, 50%WFPS and 80%WFPS were included in the experimental set up to test this objective. The presence of air dried treatments in the current experiment was important as related data on microbial communities to compare with is often available for air dried soils. Air drying was
Adapting gamma irradiation

particularly important to be included as nematodes can enter anhydrobiosis to survive drying and may regenerate during rewetting (McSorley, 2003). However, mixed effects of air drying and irradiation were observed in AIRDRIED treatments that made comparisons against 50% and 80% WFPS difficult. Air drying by itself may have effects on the soil organisms and the physico-chemical properties of the soil. For this reason, detailed comparisons were made between 50 and 80% WFPS treatments to answer the first research question.

The other objective was to check whether leaching the irradiated samples with water immediately after irradiation reduces nutrients in the flush (particularly nitrogen). Accordingly, the main findings are discussed separately in relation to each objective as a sub topic.

2.4.1 Effects of increasing moisture content at the time of gamma irradiation on nematode abundances and soil biochemical parameters

2.4.1.1 Nematode abundances

The number of living nematodes extracted at each sampling time decreased in all irradiated samples as compared to the unirradiated control regardless of the moisture content at the time of irradiation. This is, in fact, expected as the gamma rays of each dose directly damage the DNA of the cells of nematodes and prevent further reproduction of nematodes. At each dose of gamma irradiation, more nematodes were also killed in 80%WFPS than 50%WFPS treatments, confirming that the lethal effect of a given dose increases as the moisture content of the soil increases at the time of irradiation. This could be a result of indirect effects of gamma irradiation, that is, the presence of more water molecules in 80%WFPS treatments may have enabled the given dose to produce more radicals, which most likely have resulted in more nematode cell DNA damages than in 50%WFPS.

Air drying eliminated nematodes regardless of the irradiation doses throughout the incubation period and in the unirradiated CTR as well. The elimination of nematodes in AIRDRIED samples can be considered as the combined effect of air drying and irradiation doses. There were some nematodes surviving in the unirradiated CTR particularly at the beginning of the incubation. However, they were all killed as the dose increased, suggesting the direct killing effect of gamma irradiation on the surviving nematodes. Here, the indirect
effect is negligible as these air dried samples contain very little water at the time of gamma irradiation. Nematodes that may have entered the anhydrobiosis phase to survive desiccation did not regenerate during the entire incubation period.

To the best of our knowledge, no previous studies compared the effect of a given gamma irradiation dose on nematode abundance at different moisture contents. However, different authors reported contrasting effects of a given dose at a given moisture content. For example, Popenoe and Eno (1962) reported that 2.5 kGy completely eradicated free-living nematodes from sandy soil with a moisture content of 10%, in 14 days of incubation after irradiation. Buchan et al. (2012) compared the response of nematodes and microbial biomass to a range of gamma irradiation doses on moist (the exact moisture content was not reported) sandy loam and found that 5 kGy was sufficient to remove all free-living nematodes within 14 days of incubation after irradiation. Thompson (1990) reported that a 7.5 kGy dose was sufficient to entirely kill a root lesion nematode species (Pratylenchus thornei) after irradiation in air dried vertisols. The possible reason for the variability of the killing effect of each dose in these previous studies could be the differences in soil moisture content at the time of irradiation. In contrast to the recent recommendation by Buchan et al. (2012), 5 kGy dose was not sufficient to eliminate all free-living nematodes in 50%WFPS treatments in this experiment. This could probably be because of the lower actual dose applied in the current experiment (4.5 kGy) and moisture content differences between the current and previous report as moisture content of the soil at the time of irradiation was not considered in Buchan et al. (2012).

2.4.1.2 Microbial biomass C and community structure

Microbial biomass carbon ($C_{mic}$) measurements by fumigation and extraction method resulted in much higher organic C in non-fumigated than fumigated samples in AIRDRIED treatments in the CTR and at 1 kGy. Therefore, the data for $C_{mic}$ treatments was not presented here; instead the total PLFA was used as a proxy for microbial biomass carbon. In previous experiments, a significant positive correlation was found between total PLFA and $C_{mic}$ on similar soil samples collected from the same field (Buchan et al., 2013; Gebremikael et al., 2014b).
In contrast to our expectation, increasing the moisture content from 50% WFPS to 80% WFPS did not significantly change the concentration of total PLFA in general at the beginning and the end of the experiment (Figure 2.2). However, in terms of microbial community structure, PC1 segregated the unirradiated CTR samples and 1 and 3 kGy doses of 80%WFPS treatments from the rest of the treatments at t1 (Figure 2.3). According to the loadings, biomarker PLFAs of the bacterial groups (G- and G+), actinomycetes, AMF and fungi significantly contributed to this difference. Although the reason is not clear, the microbial community structure of 80%WFPS treatments receiving 3 kGy dose was very comparable to the unirradiated CTR according to PC1.

AIRDRIED and 80%WFPS treatments were consistently separated from that of LEACHED and 50%WFPS regardless of irradiation by PC2 at the beginning of the incubation and PC1 at the end of the incubation. This separation could be explained by the physical disturbance that likely occurred during wetting and air drying of both the AIRDRIED and 80%WFPS treatments before incubation. Physically protected organic matter may have become more available for microbial attack during these physical disturbances in AIRDRIED and 80%WFPS compared to 50%WFPS and LEACHED treatments (Fierer and Schimel, 2002). PC1 was significantly loaded by many of the PLFA biomarkers suggesting that sufficient substrates might have become available for all microbes.

Normally in gamma irradiated soils, the total PLFA is expected to decrease as gamma irradiation kills the soil organisms depending on the dose in comparison to unirradiated CTR. However, higher total PLFA was found in AIRDRIED treatments in irradiated soil than in the CTR (Figure 2.2). The explanation for this could be that the rate of organic matter decomposition during irradiation could be higher in the AIRDRIED soils as physically protected organic matter is made available for degradation by the gamma rays during irradiation. The carbon released in this way could be used as an energy source for the surviving microbes in the AIRDRIED soil resulting in an increase in microbial population as compared to the unirradiated CTR (McLaren, 1969; McNamara et al., 2003). At the end of the incubation, there were no significant differences between the CTR and irradiated samples in AIRDRIED
Adapting gamma irradiation treatments, suggesting that the C in the flush in the irradiated samples influenced the microbes temporarily.

### 2.4.1.3 Enzyme activities

Dehydrogenase activity is considered as an indicator of an overall biological activity in the soil (Benitez et al., 2006; Moeskops et al., 2010). In line with our expectation, dehydrogenase activity was reduced significantly as the moisture content of the soil increased from 50 to 80% WFPS in the CTR and all irradiation doses. Reduction in dehydrogenase activity indicates the death or depression of the microbial biomass in the soil most likely as a result of the indirect effect of irradiation (radiolysis of water). In AIRDRIED treatments, dehydrogenase activity increased rapidly during most of the incubation period in the CTR and 1 kGy dose samples, which seems logical as in these two treatments the microbes were not affected and their growth might have been triggered by the availability of more substrate from the air drying and rewetting process. At higher doses; however, AIRDRIED treatments did not show significant differences in dehydrogenase activity compared with moist soil treatments.

Betaglucosidase is an enzyme that is involved in the degradation of organic matter. No significant differences were observed between 50 and 80% WFPS during most of the incubation period. It is an extracellular enzyme produced by plants, nematodes and microbes, with fungi being the primary source (Esen, 1993), and mainly bound to and protected by soil colloids (Lensi et al., 1991). Because fungi are generally more sensitive than bacteria to gamma irradiation (Buchan et al., 2012; McNamara et al., 2003; Popenoe and Eno, 1962), death of more fungal biomass was expected in 80%WFPS compared to 50%WFPS due to radiolysis, which might have had effects on the enzyme activity. However, the fungal biomarker showed no significant differences between these treatments (data not shown). Betaglucosidase activity was the lowest in AIRDRIED treatments throughout the incubation period in line with previous reports by Lensi et al. (1991), who found lower betaglucosidase activity in dry soil than in wet soil. Possibly air drying dispersed soil colloids and might have exposed the bounded enzymes to be attacked by the gamma rays eventually decreasing its activity.
2.4.1.4 Evolution of soil mineral N

Increasing the water content from 50 to 80% WFPS at the time of irradiation increased ammonification which could also be attributed to the indirect effect of irradiation. In several previous reports irradiation has caused a significant ammonium flush (Buchan et al., 2012; Gebremikael et al., 2014b; Lensi et al., 1991; Singh and Kanehiro, 1970). Here, the increase was mostly during the first 42 days of incubation, suggesting that the readily degradable compounds from the dead cells and organic matter declined as the incubation proceeded. AIRDRIED treatments consistently showed significantly higher NH$_4^+$-N concentration than all other treatments in the CTR and at 3 and 5 kGy doses. Possibly, this was a combined effect of gamma irradiation and drying and wetting as well. That is, in unirradiated CTR the higher concentration of NH$_4^+$-N is most likely due to the drying and wetting effect while the additional higher concentration in the irradiated samples could be due to the gamma irradiation effect.

Unlike NH$_4^+$-N, the NO$_3^-$-N concentration decreased as the moisture content increased from 50 to 80%WFPS during most of the incubation period. Regardless of the irradiation dose, nitrification did keep on increasing throughout the incubation period, which is in agreement with our previous findings (Buchan et al., 2013; Buchan et al., 2012). The response of the nitrifying bacterial communities and activity was reported to be dose dependent. Thompson (1990) reported that the application of sub-sterilizing doses (<10 kGy) did not have a significant effect on the establishment of fully effective nitrifying bacteria as a large portion of the ammonia had been converted into NO$_3^-$-N. However, at higher dose (20 kGy) extra NH$_4^+$ produced during incubation did not nitrify, indicating elimination and no redevelopment of a productive nitrifying communities at higher doses. The presence of nematodes in irradiated samples has also been consistently reported to increase nitrification (Buchan et al., 2013; Gebremikael et al., 2014b; Xiao et al., 2010). Here, nematodes were still present in a 50%WFPS treatment while entirely absent in 80%WFPS particularly at higher doses.

Despite the accumulation of high NH$_4^+$-N in the AIRDRIED treatments, nitrification was consistently the lowest in the CTR and irradiated samples. Previous works reported that an application of a 3 kGy dose of gamma irradiation inhibited up to 75% of nitrification in air
dried sandy clay loam soil (Hasson et al., 1988). The presence of high NH$_4^+$-N accumulation and low NO$_3^-$-N suggests that interactions between air drying and gamma irradiation might have resulted in nitrification inhibition in the AIRDRIED treatments.

### 2.4.2 Effects of leaching with water immediately after gamma irradiation

To investigate whether leaching with water immediately after irradiation reduces nutrients in the flush, we only considered 50%WFPS, as this was the optimal moisture content for C and N incubation experiments. Both treatments (50%WFPS and LEACHED) were similar except that the latter was leached with water immediately after irradiation by saturating and applying suction as explained in the material and method section. The aim was to reduce the amount of easily dissolved organic compounds that became available from the lysed cells and the decomposition of organic matter during irradiation. Unfortunately, the dissolved organic N was not determined, but its possible effects on nutrient flush were assessed by measuring mineral N and microbial properties.

Leaching did not significantly reduced the abundances of nematodes as compared to the 50%WFPS treatments. We expected that leaching after irradiation would reduce dissolved organic compounds which might result in the reduction of microbial biomass, indirectly reducing the abundance of microbivorous nematodes. However, this was not supported by the total PLFA data as there was no significant difference in the total PLFA between 50%WFPS and LEACHED samples. Dehydrogenase activity was lower in the LEACHED samples than in 50%WFPS, particularly in the samples irradiated with 3 kGy and 5 kGy, suggesting a lower microbial activity in LEACHED treatments. The difference in betaglucosidase activity between these two treatments was not consistent and highly variable over the incubation period.

In agreement with our hypothesis, leaching with water immediately after irradiation resulted in significantly lower NH$_4^+$-N concentration in the irradiated samples, particularly after 42 days of incubation as compared to the 50%WFPS treatments. As discussed earlier, the substrate for ammonification during the early stages of incubation after irradiation is dissolved organic compounds from the lysed cells and organic matter decomposed by gamma irradiation. Therefore, leaching with water might have reduced these organic compounds, and eventually reduced ammonification. However, the NH$_4^+$-N concentration was still high in
the LEACHED samples as compared to the unirradiated CTR, suggesting leaching with water was not sufficient to reduce these compounds and NH$_4^+$-N concentration to the level comparable to the unirradiated CTR. As we expected NO$_3^-$-N concentration was significantly lower in LEACHED samples than in 50%WFPS because nitrate is readily leached with water. As a result of decreased NH$_4^+$-N and NO$_3^-$-N concentration in LEACHED samples, the total mineral N was also significantly lower in LEACHED samples than in 50%WFPS samples. In the current study the soil columns were oversaturated and leached only once. In other related studies (Alphei et al., 1996; Koller et al., 2013a; Koller et al., 2013c) the soil was mixed with sand (1:1 ratio) and washing was done for extended periods (e.g. five weeks) or with much more water (three fold volume). Probably, doing several leaching could have resulted in a more significant depletion of the nutrients in the flush.

2.4.3 Which dose-moisture combination should be recommended to create nematode free soil samples

The findings of this experiment show that the efficiency of a given dose of gamma irradiation in eliminating nematodes varies depending on the moisture content of the soil at the time of irradiation. Therefore, a particular dose-moisture combination needs to be selected from the treatments tested above. Presumably the first criteria should be the elimination of nematodes from the soil. In this case, the AIRDRIED, 3 kGy at 80%WFPS and 5 kGy at 80%WFPS were the candidates. In contrast to previous findings (Buchan et al., 2012; Gebremikael et al., 2014b) 5 kGy at lower moisture content (50% WFPS) did not completely eliminate nematodes (Figure 2.1d). The other criteria considered for selection of the appropriate dose were whether it leaves soil biochemical properties such as microbial biomass and nutrient concentrations comparable to the unirradiated control. The microbial biomass C and microbial community structure (as determined by PLFA) in AIRDRIED treatments were comparable to the 80% WFPS treatments, and both were also comparable to the CTR (Figure 2.2 and Figure 2.3 ), but air drying resulted in several unwanted side effects on nutrient flush and enzymatic activities. For these reasons, AIRDRIED treatments were not selected. The selection between 3 kGy and 5 kGy at 80% WFPS was made based on the PLFA data (which indicates microbial biomass and community structure). No significant differences were
generally found in the total PLFA concentration between unirradiated CTR and irradiated samples with 3 kGy at 80% WFPS at the start and end of the incubation. The total PLFA was significantly lower in samples irradiated with 5 kGy at 80% WFPS at the start of incubation. Perhaps, 3 kGy at 80% WFPS significantly affected some of the microbial groups such as actinomycetes and AMF, however, G+, G-, fungi and the B:F ratio generally were not significantly affected.

2.5 Conclusions

Increasing the moisture content at the time of irradiation increased the lethal effect of a given irradiation dose on nematodes as a result of radiolysis, which is the indirect effect of gamma irradiation. This shows the importance of moisture content of a soil sample to optimize gamma irradiation as a tool in selective sterilization or defaunation. In terms of complete eradication of nematodes, air drying is the most effective, but resulted in significant changes in several biochemical properties compared to the unirradiated control soil. The application of 3 kGy at 80% WFPS and 5 kGy at 80% WFPS eliminated nematodes. Because the total PLFA concentration and some of the microbial groups such as G+, G- and fungi in the 3 kGy at 80% WFPS showed no significant differences with unirradiated CTR, it was forwarded as a recommended dose to eliminate nematodes without significantly altering the microbial biomass and community structure. Leaching with water after irradiation significantly decreased total mineral N in the flush, but \( \text{NH}_4^+ \) concentration remained relatively high. To further reduce \( \text{NH}_4^+ \) level to the level comparable to the control, additional methods such as increasing the frequency of leaching with water, leaching with salt solutions or waiting until the \( \text{NH}_4 \) changed to nitrate and remove it by leaching need to be tested.
Chapter 3:

Quantifying the contribution of the entire free-living nematode community to carbon mineralization under contrasting C availability
Abstract

Several studies reported that nematodes respire most (31-75%) of the carbon assimilated and contribute significantly to C mineralization in terrestrial ecosystems. However, most of these findings were based on either simplified experimental setups using a few selected nematode species and bacteria as a food source or on indirect estimation using O₂ consumption and the fresh weight of nematodes. In order to understand the role of nematodes in OM decomposition, experimental set ups should include the entire nematode community and native microbes as a food source. However, there is no data on the contribution of the entire nematode community on C mineralization. To quantify their contribution we set up a long term incubation experiment and determined CO₂ evolution over time and other related parameters at the end of the incubation. The following treatments were compared with and without grass-clover amendment: defaunated and reinoculated with the entire free-living nematode community (+Nem) and defaunated and not reinoculated (-Nem). We also included untreated fresh soil as a control (CTR). Nematode abundance and diversity in +Nem was comparable to the CTR showing the representativeness of reinoculation to the field condition. No significant differences in C mineralization was found between +Nem and –Nem treatments of both the amended and unamended samples at the end of the incubation. Other related parameters such as microbial biomass (Cₘ𝑖𝑐) and enzymatic activities did not show significant differences between +Nem and –Nem treatments of both in amended and unamended samples. Total mineral N was significantly higher in +Nem treatments of the unamended soil. These findings show that the collective contribution of the entire nematode community to C mineralization is negligible. Previous reports in literature based on simplified experimental setups and indirect estimations are contrasting with the findings of the current study and should be interpreted carefully. Given the presence of several species of nematodes and a wide array of interactions amongst nematodes and microbes, such realistic experimental set ups may be applied to increase our understanding of the role of soil fauna in ecological processes.
3.1 Introduction

The three primary energy or organic matter (OM) decomposition pathways that exist in the soil food web, i.e., the bacterial, fungal and plant channels are regulated by free-living nematodes (Ferris et al., 1997; Moore et al., 2003; Zhao and Neher, 2014). For example, the fastest decomposition pathway in which bacteria are the primary decomposers, OM decomposition, is affected by bacterivorous and omnivorous nematodes as they feed on and disperse these microbes (Beare, 1997; de Ruiter et al., 1994; Freckman, 1988). Plant-root feeding and fungal feeding nematodes regulate the root and fungal energy channels, respectively, as they pierce the cell wall of plants or hyphae, during which process part of the cell contents is transferred to the labile organic matter pool (Ferris et al., 1997). Two main mechanisms for energy and nutrient flow in the soil food web, involving nematodes, have been reported: 1) C:N ratio differences between nematodes and their prey, particularly bacteria (Anderson et al., 1981; Freckman, 1988; Woods et al., 1982) and 2) high respiration efficiencies, wherein most of the assimilated C is respired as CO$_2$ (Chen and Ferris, 1999; Ferris et al., 2012; Freckman, 1988; Sohlenius, 1980).

High respiration efficiencies have consistently been reported for several nematode species in various feeding groups, indicating that it may be the main mechanism how nematodes are involved in energy and nutrient flows in soils. For instance, Wasilewska and Paplinska (1976) as cited in Sohlenius (1980) reported an average of 75% respiration efficiency for the total nematode community (ranging from 78-85% for separate nematode feeding groups) in Poland meadow ecosystems. Yeates (1973a) estimated monthly respiration efficiencies for 29 nematode species of different feeding groups in a Danish beech forest during one year and reported an average respiration efficiency of around 80%.

Despite such a high respiration efficiency of the nematode community, studies reported that the contribution of nematodes to total soil heterotrophic respiration is low. For instance, Kitazawa (1977) estimated that nematodes contribute 0.8% and Reichle (1977) 2% of the total heterotrophic soil respiration in a Japanese coniferous and US deciduous forest, respectively. In these and other related studies, the relative contribution of nematodes have been determined based on the amount of carbon nematodes assimilated and liberated as CO$_2$. 

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(Reichle, 1977; Sohlenius, 1979, 1980). However, these respiration values are not often determined by measuring CO₂ experimentally. Respiratory values have rather been estimated using the relation between the estimated weight of individual nematode population, their oxygen consumption and estimated values of the respiration quotient (RQ) (Yeates, 1973b). Thus, the reported low contribution of nematodes to the total heterotrophic soil respiration may be less accurate because of these methodological limitations.

In contrast to the above estimates, studies which measured the relative CO₂ respired by nematodes in soil microcosms reported significantly higher contribution of nematodes to C mineralization (Coleman et al., 1977; Coleman et al., 1978b). For instance, Coleman found that the presence of a bacterivorous nematode increased cumulative CO₂-C mineralized by 50% and 27% over the presence of bacteria only in glucose amended and unamended treatments, respectively. Such findings based on a single species of nematodes and bacteria does not represent the field condition where many species of nematodes interact amongst each other and with a multitude species of microbes. Moreover, the contribution of individual species to nutrient cycling may vary considerably as each nematode taxon exhibits a wide range of metabolic activity, and their energetics may depend on the food availability (C and N) and growth stage of the nematodes (Ferris et al., 1995).

Thus, the contribution of the total nematode community needs to be determined in an experimental setup that allow variations in C and N availability and several interactions between different species of nematodes and microbes. Findings based on such kind of realistic experimental setups may give more realistic and accurate estimates which increase our understanding of the role of these nematodes in C mineralization. To the best of our knowledge, there are no data on the collective contribution of the entire nematode community to C mineralization based on measured differences in CO₂-C production between treatments with and without nematodes. To do so, we set up an incubation experiment and measured CO₂ evolution in defaunated samples with or without nematodes for six months in both bare and grass-clover amended soils. The main objective of this experiment was to quantify the contribution of the entire free-living soil nematode community to C mineralization from indigenous soil organic matter and grass-clover amendment under a
realistic experimental setup. We hypothesized that free-living nematodes collectively respire more CO$_2$ in grass-clover amended microcosms in comparison to the unamended microcosms as the additional C and N fuels microbial growth which in turn stimulates the metabolic activities of the nematode community and increase C mineralization.

3.2 Materials and methods

3.2.1 Sample collection and preparation

Composite soil samples were collected from the 0-15 cm layer of an organically managed agricultural field at Merelbeke, Belgium sown with a grass-clover mix. Several augerings were collected in a zigzag pattern throughout the field after making plant free patches by gently cutting the grass-clover at the base of the plant. At the time of sampling the soil was characterized by a bulk density of 1.49 Mg m$^{-3}$, 1.23% organic C, 0.10% total N, and 22.3 µg mineral N g$^{-1}$ dry soil. The soil texture was sandy loam. A bulk sample of fresh grass-clover was also collected from the same field, chopped into small pieces and used as organic amendment in the experiment.

The composite sample was gently sieved on a 5 mm mesh in order to homogenize it and remove stones and visible soil animals such as earthworms. Part of the composite soil was transferred into 4 PVC columns (height = 30 cm and diameter =10.8 cm) each containing about 3 kg fresh soil and a bulk density of 1.40 Mg m$^{-3}$ was reconstructed. The remaining part of the composite soil sample was kept for nematode extraction. An appropriate amount of chopped fresh grass-clover was weighed and mixed into two of the PVC columns assigned for amended treatments at a rate of 4.96 tons DM ha$^{-1}$. The remaining two PVC columns that did not receive grass-clover were used for unamended treatments.

The moisture content of the soil in each PVC column was then adjusted to 80% water filled pore space (WFPS) by adding demineralized water as explained in the previous Chapter. Two of the PVC columns were subjected to a 3 kGy dose of gamma irradiation at the Synergy Health sterilizing company, Etten-Leur, The Netherlands. The exact dose applied to each PVC column was measured by a dosimeter and the average dosimeter readings were 3.0 kGy. The non-irradiated soil in the other 2 PVC columns (which was used as control) was kept in a cold room (4°C) for a day. The moisture content of the samples which was 80% WFPS at the time
of irradiation was reduced to slightly lower than 50% WFPS immediately after irradiation by spreading the soil from each PVC column separately at room temperature. (Figure 3.1)

**Figure 3.1. Schematic representation of the procedure followed to prepare the composite soil sample for gamma irradiation and for incubation. Large size PVC tubes were used for irradiation and small sized cores for incubation.**

### 3.2.2 Treatments and experimental setup

A total of 18 experimental units (six treatments in three replicates) was prepared by filling PVC cores (height = 7.5 cm and diameter = 7.5 cm) with 400 g of the respective amended and unamended soil (6 cores with control soil and 12 cores with irradiated soil) and gently compacted to a bulk density of 1.4 Mg m⁻³ (Figure 3.1). Half of the irradiated samples (n=6) were reinoculated with concentrated nematodes suspensions (about 10 ml) extracted from the same amount of fresh soil (i.e., from 400 g soil). Each nematode suspension was mixed into the corresponding PVC cores through adding several drops with a separate pipette and gently mixing before filing the PVC cores. All the samples were incubated in an incubation chamber at 17°C for six months. At the end of the incubation the following parameters were determined: mineral N, microbial biomass carbon (Cmic), phospholipid fatty acids (PLFA), dehydrogenase activity, betaglucosidase activity. The following treatments were compared with and without grass-clover amendment: 1) unirradiated fresh soil (CTR), 2) irradiated and without nematodes (-Nems) and 3) irradiated and reinoculated with nematodes (+Nems) (Figure 3.2).
3.2.3 Nematode extraction and identification

Nematode counting and identification was done at the start of the experiment for each replicate of the CTR and +Nem samples in order to check whether reinoculation was successful both in terms of nematode abundance and diversity in comparison to the unirradiated CTR. At the end of the experiment the abundance of nematodes was determined for each treatment, but no identification was done. Nematode extraction, counting and identification was carried out according to the procedure explained in detail in Buchan et al. (2013). Briefly, all free-living nematodes were extracted from 100 g moist soil (CTR and +Nem samples) using an automated zonal centrifuge machine which separates all free-living nematodes from mineral and most organic soil constituents based on their specific density (Hendrickx, 1995).

After extraction, the nematode suspension was poured onto a sieve covered with a filter paper and left for 72 hours so that only viable nematodes migrated towards the water and were collected in the bottom of the plate. All viable nematodes that passed through the filter
were then concentrated and counted using a dissecting microscope (40x). Mass fixation was done by adding hot 4% formaldehyde (65°C) at a ratio of 1:2 (5 ml nematode suspension and 10 ml formaldehyde) and immediately cooling in cold water according to the procedure by van Bezooijen (2006). After six weeks of storage at room temperature, the formaldehyde was tapped off carefully until 1 ml of the nematode suspension remained into which glycerin was added in 1:1 ratio. Slides were made for each sample and at least 125 individual nematodes were identified from each slide to family or when possible to genus level based on Bongers (1994) and assigned to the corresponding functional groups according to Yeates et al. (1993a). However, *Filenchus* was considered as fungivorous as suggested by other authors (Buchan et al., 2012; Christensen et al., 2007; Okada et al., 2005; Okada et al., 2002) and the remaining genera of the Tylenchidae family considered as root feeders. At the beginning of the experiment nematodes were extracted from the unirradiated control soil in order to compare it with the irradiated samples in terms of abundance and diversity.

### 3.2.4 **C mineralization**

Each soil core was placed inside a glass jar that was sealed air tight. A small vial containing 15 ml of 0.5M NaOH was also placed on top of the soil core in order to trap CO$_2$ mineralized from the soil. C mineralization was monitored during six months of incubation by measuring the CO$_2$ trapped in the NaOH containing vial through back titrating the excess NaOH with 0.2M HCl after precipitating the carbonates with BaCl$_2$. During titration, the jars were left open for about 3 hrs to replenish the soil with oxygen. Throughout the incubation CO$_2$ was determined at 23 sampling points each time from 3 replicates. Initially CO$_2$ was measured every day during the first 72 hours and sampling intensity was gradually changed to every three days, every week and finally to every two weeks as the rate of C mineralization was decreasing.

The cumulative amount of CO$_2$ produced in each treatment was calculated for both amended and unamended samples. The cumulative amount of CO$_2$ produced from the added grass-clover amendment was determined by considering the simple difference between the cumulative CO$_2$ evolved from the grass-clover amended treatments and the cumulative CO$_2$ evolved from the equivalent treatment in the unamended soil. The cumulative C mineralized (Ct) was then plotted against incubation time (t). C (t) was further fitted to a
parallel first order plus zero order kinetic model according to the Levenberg-Marquardt algorithm:

\[ C(t) = C_0 (1 - \left[ e^{xp(-k_f * t)} \right] + k_s * t) \]

This model assumes the existence of two pools of available C with different resistance against microbial degradation; an easily mineralizable pool and a more resistant C pool (Sleutel et al., 2005). The former pool mineralizes according to first order kinetics at a rate constant of \( k_f \) \( \text{day}^{-1} \) and the latter pool mineralizes according to zero order kinetics at a rate of \( k_s \) (\( \mu g \ CO_2-\text{C} \ \text{g}^{-1} \ \text{soil day}^{-1} \)). The validity of the model was evaluated by the standard error of the parameters and correlation between the parameters. According to Hess and Schmidt (1995) and Sleutel et al. (2005), C mineralization models can be considered valid only when the standard errors of the parameter estimates are smaller than 50% of the parameter values.

3.2.5 Microbial Biomass C and Phospholipid fatty acids (PLFA)

Microbial biomass carbon was determined by the fumigation extraction technique using the procedure by Vance et al. (1987) at the end of the incubation. Changes in the structure of the microbial community were determined based on microbial membrane phospholipid fatty acids (PLFA) following a method explained in details by Buchan et al. (2013). Briefly, 4 g of freeze-dried soil from each sample was sieved (2 mm) in order to homogenize and remove the root fragments and stones. Phospholipids were extracted from this freeze-dried soil and transformed into methyl esters (FAMEs). Finally, individual FAMEs were identified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS) on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Interscience BVBA, Louvain-la-Neuve, Belgium) in the electron ionization mode.

The sums of marker fatty acid concentrations for selected microbial groups were calculated as follows. For Gram-positive bacteria the sum of i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0; for Gram-negative bacteria cy17:0, cy19:0, C16:1ω7, C16:1ω9; for the actinomycetes the sum of 10-methyl branched saturated fatty acids (Buyer and Sasser, 2012; Frostegard et al., 1996; Kaiser et al., 2010). For the total bacterial community the fatty acids 15:0, 17:0 and cy19:0 were also included in addition to the sum of marker PLFAs for Gram-positive bacteria,
Gram-negative bacteria and actinomycetes. For saprotrophic fungi the marker PLFAs 18:2ω6c and 18:1ω9; and for arbuscular mycorrhizal fungi (AMF) 16:1ω5c were considered (Joergensen and Wichern, 2008). The bacterial: fungal ratio was calculated as the sum of total bacterial marker fatty acids divided by the saprotrophic fungal marker fatty acids.

3.2.6 Enzymatic activities

Dehydrogenase and betaglucosidase enzyme activities were determined at the end of the incubation according to the same procedure used in the previous Chapter (2.2.7).

3.2.7 Mineral N

Nitrogen mineralization was determined by measuring the evolution of mineral nitrogen ($\text{NH}_4^+$ and $\text{NO}_3^-$) throughout the incubation. Both $\text{NH}_4^+$ and $\text{NO}_3^-$ were measured from the same aliquot colorimetrically in a continuous flow auto analyzer (Chem-lab 4, Skalar 223 Analytical, Breda, The Netherlands), following the extraction of 30 g moist soil with 150 ml 1M KCl (1:5 ratio) after shaking for one hour.

3.2.8 Statistical analysis

The experiment followed a factorial design with two fixed factors. The first factor is amendment and had two levels: grass-clover amended and unamended. The second factor nematode had three levels: i) non irradiated fresh soil (CTR), ii) defaunated and not reinoculated (-Nem) and iii) defaunated and reinoculated with the entire free-living nematode community (+Nem). Statistical analysis was conducted accordingly using a two way analysis of variance (ANOVA) model after checking the assumptions for homoscedasticity and normality of all the variables. Data transformation (log or sqrt) was done for the variable that violated the assumptions for ANOVA. Whenever the interaction between amendment and treatment was non-significant ($p>0.05$), the main effects were compared and Fisher’s least significant difference (LSD) was used to analyse mean differences. However, when the interaction term was significant ($p<0.05$), one-way ANOVA model was fitted for each time-treatment combination and tukey’s method was used for the post hoc mean difference analysis. The PLFA data were further analyzed by Principal Component Analysis (PCA) based on the correlation matrix after checking the sampling adequacy using the Kaiser-Mayer-Olkin
(KMO) test. All statistical analysis was carried out using IBM SPSS Statistics 20 software (SPSS inc., Chicago, USA).

3.3 Results

3.3.1 Nematode abundance and diversity

At the beginning of the incubation, all the nematode taxa identified in the CTR samples were also found in the +Nem samples (Table 3.1). Nematode abundance in +Nem and CTR samples at the beginning of the incubation was comparable both in unamended (16.9 vs 17.8 ind.g\(^{-1}\) soil) and amended soil (18.0 vs 18.3 ind.g\(^{-1}\) soil). However, the abundance significantly decreased (p<0.05) over the incubation period in unamended soil, while it increased in amended soil (Figure 3.3). +Nem samples also showed significantly lower (p<0.05) abundance than the CTR at the end of the incubation.

\[ \text{Figure 3.3. Nematode abundances (individual nematodes per gram of dry soil) in amended and unamended soil at the beginning (t0) and end (t final) of the incubation period. The error bars are standard error of the mean (n=3).} \]
Table 3.1 Mean nematode abundances (ind. g⁻¹ soil) and standard error of the mean (n=3) in unirradiated fresh soil (CTR) and irradiated and reinoculated soil (+Nem) at the start of the incubation experiment. Feeding groups are assigned according to Yeates et al. (1993a) except Filenchus as explained in section 3.2.4.

<table>
<thead>
<tr>
<th>Family/Genus</th>
<th>Feeding groups</th>
<th>Mean abundance (individuals g⁻¹ soil) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CTR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reinoculated (+Nem)</td>
</tr>
<tr>
<td>Aphelenchoides</td>
<td>fungivorous</td>
<td>0.11±0.09</td>
</tr>
<tr>
<td>Aphelenchus</td>
<td>fungivorous</td>
<td>0.34±0.28</td>
</tr>
<tr>
<td>Cephalobidae</td>
<td>bacterivorous</td>
<td>4.68±0.21</td>
</tr>
<tr>
<td>Coslenchus</td>
<td>Plant and root</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td>Dolichodoridae</td>
<td>Plant and root</td>
<td>0.24±0.06</td>
</tr>
<tr>
<td>Dorylaimidae</td>
<td>omnivorous</td>
<td>0.36±0.20</td>
</tr>
<tr>
<td>Filenchus</td>
<td>fungivorous</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>Meloidogyne</td>
<td>Plant and root</td>
<td>0.83±0.02</td>
</tr>
<tr>
<td>Nygolaimidae</td>
<td>carnivores/predators</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Panagrolaimida</td>
<td>bacterivorous</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>Paratylenchus</td>
<td>Plant and root</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>Plectus</td>
<td>bacterivorous</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>Pratylenchus</td>
<td>Plant and root</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>Pristionchus</td>
<td>bacterivorous</td>
<td>0.11±0.09</td>
</tr>
<tr>
<td>Psilenchus</td>
<td>Plant and root</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>Rhabditidae</td>
<td>bacterivorous</td>
<td>5.93±0.32</td>
</tr>
<tr>
<td>Seinura</td>
<td>Predator</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Steinernema</td>
<td>Entomopathogenic</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Heteroderinae</td>
<td>Plant and root</td>
<td>1.95±0.19</td>
</tr>
<tr>
<td>Tylenchidae</td>
<td>Plant and root</td>
<td>2.08±0.11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>18.31±1.00</strong></td>
</tr>
</tbody>
</table>

66
3.3.2 C mineralization

Cumulative C mineralization over time tended to be higher in +Nem than in –Nem treatments throughout the incubation period in unamended soil and lower in amended soil (Figure 3.4a). At the end of the incubation, 1.95% more cumulative CO$_2$-C was found in +Nem treatment of the unamended soil. In amended soil, however, 2.47% lower cumulative CO$_2$-C was found in +Nem compared to –Nem. The differences in CO$_2$-C between +Nem and –Nem at the end of the incubation were not statistically significant (p>0.05) both in amended and unamended soils. At the end of the incubation, the mean cumulative C mineralization was significantly higher (p<0.05) in the CTR than in both irradiated +Nem and –Nem treatments of the unamended samples. No significant differences were found between the CTR and nematode treatments in the amended soil.

Net C mineralization from the grass-clover amendment, was calculated as the simple differences in each treatment between amended and the corresponding unamended soil (Figure 3.4b). At the end of the incubation period, 48.7% (±1.93), 45.3% (±0.37) and 26.9% (±0.99) of the amended C was mineralized in –Nem, +Nem and CTR treatments, respectively. The net cumulative C mineralized from the amendment was not statistically different (p>0.05) between +Nem and -Nem treatments.

Statistically significant interaction (p<0.01) was found between the amendment and nematode treatments for all parameters of the first plus zero order kinetic model at the end of the incubation. The model parameters generally followed similar trends as measured cumulative C mineralized. No statistical differences were found between +Nem and –Nem treatments in the estimated readily mineralizable C pool ($C_0$), the mineralization rates of both the readily mineralizable pool ($K_r$) and the slowly mineralizable pool ($K_s$)(Table 3.3). The exception to this was $K_r$ in amended soil, which was found to be significantly lower in +Nem than in –Nem. Like the measured cumulative C mineralized at the end of the incubation, the estimated mean cumulative C mineralized was not significantly different between +Nem and –Nem treatments of both the amended and unamended samples (Table 3.3). The unirradiated CTR samples showed significantly different outputs (p<0.01) for all the model
parameters compared to both –Nem and +Nem treatments of the amended and unamended samples.

**Table 3.2** P values of the selected parameters after analysis of two way ANOVA at the end of the incubation for the factors treatment (CTR, +Nem, -Nem) and amendment (Amended and unamended). Further P values are given below for the cumulative CO$_2$-C measured and outputs of the model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Amendment</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PLFA</td>
<td>0.000</td>
<td>0.000</td>
<td>0.548</td>
</tr>
<tr>
<td>C mic</td>
<td>0.022</td>
<td>0.029</td>
<td>0.001</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>0.005</td>
<td>0.672</td>
<td>0.091</td>
</tr>
<tr>
<td>Betaglucosidase</td>
<td>0.000</td>
<td>0.010</td>
<td>0.117</td>
</tr>
<tr>
<td>NH$_4$-N</td>
<td>0.169</td>
<td>0.047</td>
<td>0.129</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>0.003</td>
<td>0.000</td>
<td>0.073</td>
</tr>
<tr>
<td>Total mineral N</td>
<td>0.004</td>
<td>0.000</td>
<td>0.085</td>
</tr>
<tr>
<td>Cumulative C measured</td>
<td>0.830</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Cumulative C model estimated</td>
<td>0.865</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>C$_0$</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>K$_f$</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>K$_s$</td>
<td>0.003</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR Vs +Nem</th>
<th>CTR Vs -Nem</th>
<th>+Nem Vs -Nem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cum. C measured</td>
<td>UNA 0.001</td>
<td>0.002</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>Am 0.000</td>
<td>0.000</td>
<td>0.743</td>
</tr>
<tr>
<td>Cum. C model</td>
<td>UNA 0.004</td>
<td>0.001</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>Am 0.000</td>
<td>0.000</td>
<td>0.745</td>
</tr>
<tr>
<td>C$_0$</td>
<td>UNA 0.000</td>
<td>0.000</td>
<td>0.932</td>
</tr>
<tr>
<td></td>
<td>Am 1.000</td>
<td>0.000</td>
<td>0.933</td>
</tr>
<tr>
<td>K$_f$</td>
<td>UNA 0.000</td>
<td>0.000</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>Am 0.000</td>
<td>0.000</td>
<td>0.029</td>
</tr>
<tr>
<td>K$_s$</td>
<td>UNA 0.000</td>
<td>0.000</td>
<td>0.805</td>
</tr>
<tr>
<td></td>
<td>Am 0.991</td>
<td>0.997</td>
<td>0.908</td>
</tr>
</tbody>
</table>
Figure 3.4. a) Cumulative C mineralization (µg CO₂-C g⁻¹ soil) as a function of the incubation time. b) Cumulative C mineralized (µg CO₂-C g⁻¹ soil) from the grass-clover amendment calculated as the difference between amended and unamended treatments over time. Vertical bars indicate standard error of the mean (n=3).
Table 3.3 Parameters of the first- plus- zero order kinetic model fitted to the cumulative C mineralization data of each treatment (n=3 and standard error in parenthesis). C0 is the size of the readily mineralizable C pool; ks and kf are the mineralization parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C0 (µg C g⁻¹soil)</th>
<th>kₖ (Day⁻¹)</th>
<th>Kₖ (µg C g⁻¹ soil day⁻¹)</th>
<th>Cumulative CO₂-C (µg C g⁻¹ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unamended</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNACTR</td>
<td>885.9 (211.5)</td>
<td>0.009 (0.002)</td>
<td>0.65 (0.70)</td>
<td>865.8 (12.2)</td>
</tr>
<tr>
<td>UNA-Nem</td>
<td>286.1 (6.9)</td>
<td>0.035 (0.001)</td>
<td>2.27 (0.05)</td>
<td>723.6 (7.2)</td>
</tr>
<tr>
<td>UNA+Nem</td>
<td>290.8 (10.1)</td>
<td>0.038 (0.002)</td>
<td>2.32 (0.07)</td>
<td>739.1 (9.0)</td>
</tr>
<tr>
<td><strong>Grass-clover amended</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmCTR</td>
<td>683.6 (85.4)</td>
<td>0.041 (0.009)</td>
<td>3.63 (0.58)</td>
<td>1270.5 (23.0)</td>
</tr>
<tr>
<td>Am-Nem</td>
<td>740.0 (21.7)</td>
<td>0.054 (0.003)</td>
<td>3.54 (0.16)</td>
<td>1423.8 (33.7)</td>
</tr>
<tr>
<td>Am+Nem</td>
<td>735.4 (13.4)</td>
<td>0.048 (0.002)</td>
<td>3.39 (0.10)</td>
<td>1388.7 (5.3)</td>
</tr>
</tbody>
</table>

3.3.3 Microbial community structure and biomass C

A significant interaction (p<0.05) was found between nematode treatments and amendment on Cmic, but not on total PLFA and marker PLFAs. The microbial biomass C in +Nem was not statistically different compared to −Nem treatments of both the amended and unamended soil. The CTR samples; however, showed significantly higher (p<0.05) Cmic than both +Nem and −Nem treatments of the unamended soil. In amended soil, no differences were observed between the CTR and both nematode treatments.

The concentration of total PLFA and signature PLFAs of each of the microbial groups did not show significant differences between −Nem and +Nem treatments in both amended and unamended soil (Table 3.4). Likewise, no significant differences were found in the bacteria to fungi ratio (B: F) between +Nem treatments and −Nem treatments. PCA analysis showed some differences between grass-clover amended and unamended treatments, and also between CTR and irradiated treatments (−Nem and +Nem). PC1 which explained 52% of the total variation clearly separated the CTR from both irradiated samples (Figure 3.5). PC2 which explained 16% of the total variation clearly separated the amended samples from that of unamended regardless of treatments. PC1 is highly loaded with biomarker PLFAs of several
microbial groups such as actinomycetes, G+ and G− bacteria and fungi, while PC2 is mainly loaded with non-signature fatty acids (C18:0 and C20:0) and a few signature PLFAs of bacteria and fungi (Figure 3.5; Appendix 2).

Table 3.4 Cumulative CO₂-C mineralized, microbial biomass C, and respiration rates at the end of the incubation in the amended and unamended soil. Mean values (n=3) and standard error of the mean in parenthesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative mineralized C (µg CO₂-C g⁻¹ soil)</th>
<th>Microbial biomass C (µg C g⁻¹ soil)</th>
<th>Respiration rate (µg CO₂-C g⁻¹ soil day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unamended</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>866.9 (12.5)</td>
<td>241.24 (11.06)</td>
<td>4.49</td>
</tr>
<tr>
<td>-Nem</td>
<td>717.1(6.9)</td>
<td>128.43 (26.59)</td>
<td>3.72</td>
</tr>
<tr>
<td>+Nem</td>
<td>731.1(9.3)</td>
<td>172.51 (6.87)</td>
<td>3.79</td>
</tr>
<tr>
<td><strong>Grass-clover amended</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>1244.5 (95.4)</td>
<td>198.00 (5.79)</td>
<td>6.45</td>
</tr>
<tr>
<td>-Nem</td>
<td>1398.9 (31.3)</td>
<td>230.72 (8.37)</td>
<td>7.25</td>
</tr>
<tr>
<td>+Nem</td>
<td>1364.4 (5.7)</td>
<td>195.20 (10.49)</td>
<td>7.07</td>
</tr>
</tbody>
</table>

3.3.4 Enzymatic activities

No significant (p>0.05) interactions were found between nematode treatment and amendment on both dehydrogenase and betaglucosidase activities. Both of the enzyme activities did not show significant differences (p>0.05) between +Nem and −Nem treatments in the amended and unamended samples (Figure 3.6). However, CTR samples showed significantly (p<0.05) higher enzymatic activities than both +Nem and −Nem treatments. Betaglucosidase activity was significantly higher (p<0.05) in grass-clover amended samples than in unamended samples, while dehydrogenase activity was not significantly different.
3.3.5 Mineral nitrogen

No significant (p>0.05) interactions were found between nematode treatment and amendment on mineral nitrogen concentration at the end of the incubation. +Nem treatments resulted in significantly higher NO\(_3\)-N and total mineral N concentration (p<0.05) than in both –Nem and CTR treatments of the amended samples (Figure 3.7). No significant (p>0.05) differences were observed in NH\(_4\)+-N between the nematode treatments of both amended and unamended samples. The mineral N concentrations were significantly higher (p<0.05) in amended than unamended soil.
Table 3.5 Mean concentrations of selected biomarkers for major microbial groups in nmole g\(^{-1}\) dry soil with standard error of the mean in parenthesis (n=3 ± SE). The p values are based on two-way ANOVA model.

<table>
<thead>
<tr>
<th></th>
<th>Gram+</th>
<th>Gram-</th>
<th>Actinomycetes</th>
<th>Fungi</th>
<th>protozoa</th>
<th>B:F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amendment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unamended</td>
<td>4.60±0.68</td>
<td>6.03±0.72</td>
<td>1.89±0.39</td>
<td>1.57±0.24</td>
<td>1.63±0.03</td>
<td>8.30±0.85</td>
</tr>
<tr>
<td>Amended</td>
<td>4.83±0.65</td>
<td>6.24±0.68</td>
<td>2.12±0.36</td>
<td>1.77±0.27</td>
<td>1.89±0.16</td>
<td>7.78±1.74</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>4.51±0.39</td>
<td>5.93±0.43</td>
<td>1.83±0.22</td>
<td>1.59±0.15</td>
<td>0.17±0.01</td>
<td>8.01±0.95</td>
</tr>
<tr>
<td>-Nem</td>
<td>5.02±0.79</td>
<td>6.48±0.82</td>
<td>2.02±0.49</td>
<td>1.88±0.26</td>
<td>0.20±0.05</td>
<td>7.58±2.05</td>
</tr>
<tr>
<td>+Nem</td>
<td>4.61±0.76</td>
<td>6.00±0.81</td>
<td>1.99±0.39</td>
<td>1.54±0.27</td>
<td>0.16±0.03</td>
<td>8.54±2.07</td>
</tr>
<tr>
<td><strong>p (treatment)</strong></td>
<td>0.61</td>
<td>0.72</td>
<td>0.65</td>
<td>0.32</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>p (amendment)</strong></td>
<td>0.62</td>
<td>0.74</td>
<td>0.47</td>
<td>0.31</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>p (trt*amendment)</strong></td>
<td>0.94</td>
<td>0.21</td>
<td>0.98</td>
<td>0.88</td>
<td>0.54</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Figure 3.6. The mean values of total PLFA (nmole g\(^{-1}\)soil), dehydrogenase activity (µg TPF g\(^{-1}\) day\(^{-1}\)) and betaglucosidase activity (µg PNP g\(^{-1}\) hr\(^{-1}\)) at the end of the incubation period. The error bars indicate standard error of the mean (n=3).
3.4 Discussion

The primary objective of this experiment was to quantify the collective contribution of the total free-living nematode community to C mineralization from soil organic matter and grass clover amendments over a long term incubation. The nematode contribution was determined by simple differences in CO$_2$-C mineralized between defaunated samples reinoculated with the entire free-living nematode community (+Nem) and without nematodes (-Nem), as the only difference between the two treatments was the presence or absence of nematodes. CTR treatments comprise not only nematodes but all other fauna, thus is not entirely comparable with +Nem treatments which has no other fauna except nematodes. The purpose of the CTR treatment in this experiment was to get background information on the field conditions and
evaluate the representativeness of the setup, particularly in terms of nematodes and microbial community.

### 3.4.1 Validity and representativeness of the experimental setup

Nematode respiration is regulated by several biotic and abiotic factors such as food availability (mainly C and N), the weight, size and growth stage of nematodes, the turnover rate of nematodes and ambient temperature (Ferris et al., 1995; Klekowski et al., 1972; Sohlenius, 1980). Thus, experimental setups should allow all these interactions as much as possible for a realistic determination of their role in soil respiration. In this experiment, we aimed to keep all these factors as representative as possible to field conditions.

Nematode abundance and diversity was representative of the field situation, as the native nematode taxa identified in the CTR (fresh field soil), were also found in the reinoculated soil (+Nem) (Table 3.1) and abundance was also similar in the CTR and the +Nem treatments (18.3 vs 18.1 individuals g\(^{-1}\) dry soil) at the start of the incubation. However, at the end of the incubation experiment, the abundance in +Nem treatments was significantly lower than in the CTR. The disturbance during extraction and reinoculation may possibly have reduced their survival after reinoculation. The total microbial biomass and microbial diversity in +Nem was comparable to the unirradiated CTR soil (Table 3.4). Given that bacterivores, fungivores and omnivores feed on microbes, the presence of indigenous microbes and free-living nematodes is crucial in realistic experimental setups. Previous studies considered only a few selected species of bacterial feeding nematodes (Ferris et al., 1995; Ferris et al., 1997; Marchant and Nicholas, 1974). Others did not measure respiration directly in the laboratory, but instead estimated it indirectly from individual nematode biomass (Neher and Powers, 2004; Sohlenius, 1980; Wasilewska and Paplinska, 1976).

Because the experiment was conducted over the course of around 6 months, the natural attributes of nematodes such as turnover rates may follow a comparable pattern to that found in the field. The identification showed that the +Nem samples comprised families ranging from smaller-sized nematodes, with faster turnover rates, such as *Rhabditidae* and *Cephalobidae* to large sized nematodes, with slower turnover rates such as dorylaimids (Ferris, 2010a).
Grass-clover amended samples received an additional 1398 and 130 μg C and N g\(^{-1}\) dry soil, respectively, from the amendment, showing C was not limiting. Nitrogen, an element that often limits the microbial activity in terrestrial ecosystems, was present in high concentration and was significantly higher in amended than in unamended soil (Figure 3.7) showing microbes were not nutrient limited. Likewise, the total microbial biomass and the biomass of the bacterial and fungal groups were significantly higher in amended than in unamended soil (Table 3.4 and Table 3.5).

Increasing the concentration of available C and N presumably fuels the growth of microbial biomass, which are food sources for bacterivorous, fungivorous and omnivorous nematodes. Laboratory experiments often use a bacterial species (e.g. \textit{E. coli}) to study the activity of nematodes, despite the presence of extremely diverse species of bacteria in the field (Ferris et al., 1995; Marchant and Nicholas, 1974). It has been consistently reported that the native microbial biomass can be kept largely intact by applying low doses of gamma irradiation, as was the case in the current experiment (Buchan et al., 2013; Buchan et al., 2012; Gebremikael et al., 2014b).

3.4.2 C mineralization in unamended soil

Significant interaction effects between the presence of nematodes and amendments were found on cumulative C mineralized and microbial biomass C at the end of the incubation. This means that the effect of the presence or absence of nematodes on these parameters depends on the amendment, indicating the need to compare nematode treatments with or without grass-clover amendment separately.

In unamended soil, the presence of the entire free-living nematode community did not significantly stimulate C mineralization from the native soil organic matter. Previous studies estimated that nematodes contribute between 0.3 to 2% of the total soil respiration in different forest ecosystems (Sohlenius, 1980; Yeates, 1979). The contribution of nematodes to C mineralization (about 2%) at the end of the current 6 months incubation cannot be directly compared with the above mentioned estimates because of the differences in experimental setups. Moreover, given that the differences are not statistically different in the current findings, this contribution is negligible. When assessing the effects of nematodes in a
complex system such as soil, with countless interactions, preferably a set of multiple related parameters should be used rather than relying on a single parameter. Like the cumulative C mineralized at the end of the incubation, the microbial biomass C, which regulates C mineralization and both dehydrogenase and betaglucosidase activities, which indicate microbial respiration and organic matter breakdown were not significantly different in +Nem compared to −Nem. Thus, these parameters may be considered as additional supporting indicators of C mineralization.

### 3.4.3 C mineralization in grass-clover amended soil

In contrast to our hypothesis, the amount of C mineralized from the amendment in the presence of nematodes was not significantly different compared to the treatments in their absence. Likewise, the difference in cumulative C mineralized from the soil OM and the grass-clover amendment between +Nem and −Nem treatments at the end of the incubation was not significant (Figure 3.4b). The parameters of the first-plus-zero-order model (Table 3.3) and other related parameters such as C\text{mic}, and both enzymatic activities were also not significant indicating further that the effect of nematodes on C mineralization is negligible (Table 3.4 and Figure 3.6).

There are no previous comparable experimental setups that measured CO\textsubscript{2} release from samples reinoculated with the entire free-living nematode community. Coleman et al. (1978a) reported cumulative CO\textsubscript{2}-C during 24 days of incubation in glucose amended and unamended soil samples using a single species of nematode (Mesodiplogaster) and bacteria (Pseudomonas). This nematode species + Pseudomonas treatment increased C mineralization by 350 (50%) and 145 (27%) µg cumulative CO\textsubscript{2}-C g\textsuperscript{-1} soil over bacteria only treatments in glucose amended and unamended soil respectively. In Coleman et al. (1978a), the rate of total respiration was 43.5 and 28.1 µg CO\textsubscript{2}-C g\textsuperscript{-1} soil day\textsuperscript{-1} in the presence of nematodes in amended and unamended soil, respectively. This is in contrast to the current study, which found 7.07 and 3.79 µg CO\textsubscript{2}-C g\textsuperscript{-1} soil day\textsuperscript{-1} in the presence of nematodes in amended and unamended soil respectively (Table 3.4). The respiration rates in +Nem samples were comparable to the rate of respiration in the CTR samples (6.45 and 4.49 µg CO\textsubscript{2}-C g\textsuperscript{-1} soil day\textsuperscript{-1} in amended and
unamended samples, respectively; whereas in Coleman et al. (1978a) there was no control soil to compare the results with.

Anderson et al. (1981) also found that the presence of the same species (Mesodiplogaster inheritieri) or Acrobealoides sp. significantly increased C mineralization in glucose amended microcosms as compared to similar cores with bacteria only. However, they used a very high density of nematodes, particularly for Acrobealoides sp. (96 ind. g⁻¹ soil), a density that is highly unlikely to be present in field conditions. Such results show that the contribution of a single species of nematodes to C mineralization by feeding on a single food source (bacteria) is very contrasting to the contribution of the entire nematode community in the presence of multiple food sources (native microflora).

Bacteria and fungi are the primary heterotrophic decomposers that degrade the native and the added OM. Nematodes and other microbial grazers regulate the OM decomposition as they regulate the activities of microbes. One of the mechanisms through which nematodes increase microbial activity is by transporting microbes from a region of less or no available food to substrate rich microsites (Freckman, 1988). Given the availability of sufficient and more uniformly distributed substrate and available nutrients in the amended microcosms of the current study, microbes may not have benefited from the presence of nematodes. Bjørnlund et al. (2012) suggested that, in the presence of a higher concentration of easily degradable C, the bacterial degradation may be sufficient to release the amount of nutrients required for growth, particularly N, and the contribution of microbial grazers such as nematodes could be negligible. Similar suggestion was given by Ingham et al. (1985) based on findings in amended microcosms. However, in situations of low nutrient availability, the production efficiency of nematodes may be drastically reduced and respiration may even exceed assimilation (Yeates, 1979). The findings in the current experiment indicate that the contribution of nematodes to overall C mineralization in soils is not significant, regardless of the resource availability.

Both the nematode and microbial community structure may also play important roles in the contribution of nematodes to C mineralization. The contribution of each trophic group of nematodes to C mineralization is different (Sohlenius, 1979). Previous reports indicated that
there could be a shift in nematode community structure over time depending on the availability of resources (Bongers, 1999; Ferris and Bongers, 2006). Although all the feeding groups were found in +Nem treatments at the beginning of the incubation, the evolution of their community structure over time was not determined in this experiment. The abundance of nematodes significantly increased in amended soil (Figure 3.3), but the overall abundance alone does not allow to explain succession. Data on changes in nematode community structure over time would be needed to further our understanding of the link between nematode feeding behaviour and the C mineralization process.

The principal component analysis of PLFA data showed that there was a slight shift in microbial community structure, as illustrated in Figure, wherein the CTR treatments, regardless of amendment, were separated from both +Nem and –Nem treatments at the end of the incubation by PC1, which was uniformly loaded by most of the individual PLFAs and microbial groups (Figure 3.5; Appendix 2). The uniform loadings suggest that no single microbial group dominated the CTR or the nematode treatments. This evidence was further supported by the absence of significant differences in microbial groups between CTR and nematode treatments (Table 3.4). Because irradiation took place after incorporating the amendment, it could be possible that the gamma irradiation had affected the biochemical properties of the grass clover and consequently stimulated more microbial growth and activity. This could also be the reason why C mineralization and $C_{mic}$ were higher in the CTR in unamended microcosms while it was lower in the CTR treatment of amended microcosms. PC2 that separated amended from unamended treatments was mainly loaded by the non-biomarker long chain saturated PLFAs such as C17:0 and C20:0, suggesting that microbial species with these PLFAs were segregating the amended treatment from that of unamended. This difference in microbial community structure in amended and unamended treatments accounted only for 16% of the total variation. The abundance of each microbial group was also generally higher in amended treatments than in unamended treatments (Table 3.4), suggesting that the grass clover amendment stimulated the entire microbial community, instead of shifting the microbial community structure. However, PLFA may not detect detailed changes in bacterial community. Fierer et al. (2012) applied a suite of molecular approaches to study the effects of different levels of nitrogen availability on bacterial diversity and found
a shift in bacterial community that likely led to a decrease in soil respiration at high nutrient availability.

Unlike C mineralization, mineral nitrogen was significantly higher in +Nem than in –Nem in amended treatments with a mean difference of 29.8 mg kg\(^{-1}\). As mentioned in the introduction, two direct mechanisms have been suggested to explain how nematodes contribute to N mineralization: by having a higher C:N ratio than their prey, and high respiration rates. Because of these two metabolic attributes, nematodes may excrete N in excess of their metabolic need. However, these mechanisms have only been proven for a few selected species of nematodes (Chen and Ferris, 2000; Ferris et al., 1997; Marchant and Nicholas, 1974). The findings of the current study do not support high respiration by nematodes as a possible mechanism for N excretion, because no significant effects of nematodes on respiration was observed. Given that nematodes, particularly bacterivores have higher C:N ratio than their prey, they may excrete excess N, possibly irrespective of the C mineralized. Although the C:N ratio of nematodes was not determined in the current study, differences in C:N ratio could explain the significant release of N without significant effect in C mineralization (Ferris et al., 2012). Grazing by nematodes has been shown to increase the microbial turnover that may lead to the release of N that would have otherwise been locked in the microbial biomass (Ferris et al., 2012; Griffiths, 1994; Ingham et al., 1985). Possibly, as more microbes are grazed, the immobilization of N may decrease which in turn may result in an increased availability of mineral N. This is supported by the significantly lower mineralization rate (K\(_f\)) of the easily decomposable OM pool. The other possible explanation for the increased N mineralization in the amended soil could be the low C:N ratio of the applied amendment, which may also have reduced microbial immobilization.

3.5 Conclusions

In this experiment, we studied the contribution of the entire free-living nematode community to C mineralization under realistic conditions and contrasting nutrient availabilities for the first time. The data showed that the collective contribution of the total free-living nematode community to C mineralization from SOM and grass-clover amendment was not statistically significant. Our findings contrast with previous findings in literature indicating that the results
of simplified experiments using only a few selected species of nematodes do not reliably represent a natural soil environment. The findings thus question the reliability of large values of respiration by nematodes that have been reported from experiments based on unrealistic experimental setups and estimates based on nematode biomasses. Moreover, high respiration as a mechanism to explain how nematodes directly excrete excess N is not supported by the results of this experiment, particularly in amended soil. We suggest that the difference in C:N ratio between nematodes and their prey could be responsible for the direct excretion of excess nutrients such as N.
Chapter 4:

Quantifying the contributions of nematodes to N mineralization from the SOM in bare and planted microcosms

This Chapter has been published in:

N mineralization from SOM in bare and planted microcosms

Abstract

Several ecosystem processes such as Nitrogen mineralization are mainly controlled by complex multitrophic interactions in which nematodes play major roles. Despite the abundance and diversity of these nematodes, studies on their contribution to N mineralization have been limited to a few selected species and often in completely sterilized media. Such simplified experiments are unrealistic and usually give inaccurate results as part of the interaction is missed. Therefore the contributions of nematodes to N mineralization need to be quantified more accurately in realistic conditions where native microbes, nematodes and plants interact. To do this, we set up a microcosm incubation experiment in which the whole nematode community were extracted from fresh soil and reinoculated into the soil defaunated by 5 KGY dose gamma irradiation that leaves the microbial community largely intact. Three treatments namely control, +Nem (with nematodes) and –Nem (without nematodes), with or without plants were incubated for 86 days. In bare microcosms, +Nem cores increased total mineral nitrogen with 32% compared to –Nem. However, no significant (p>0.05) differences were observed on total mineral nitrogen and plant N uptake between +Nem and –Nem treatments in planted microcosms. Nematode abundances and community structures were significantly changed over time and differed in bare and planted microcosms. Bacterivores dominated in bare, while herbivores dominated in planted soils throughout the incubation period. This could explain the contrasting effects of nematodes in N mineralization in bare and planted microcosms. Optimization of gamma irradiation technique is required to further reduce its side effects on nutrient flush and non-targeted organisms.
4.1 Introduction

Free-living soil nematodes are extremely abundant (3.5-5 million m⁻²) and diverse (33-384 species m⁻²) in terrestrial ecosystems (Neher and Powers, 2004; Yeates, 2003). Nematodes have evolved a diversity of feeding mechanisms and occupy key positions in the soil food web that enabled them to highly interact with microbes, other soil fauna, plants and among themselves, and thus contribute to the crucial ecosystem services such as nutrient cycling (Deruiter et al., 1993; Ferris, 2010a). Nitrogen (N) mineralization and N availability in the soil are mainly controlled by these complex multitrophic interactions in which nematodes of different feeding groups play direct and indirect roles. Plant-feeding nematodes increase the release of exudates into the soil stimulating more microbial growth (Denton et al., 1999; Yeates et al., 1999a; Yeates et al., 1999b). Bacterivorous and fungivorous nematodes feed on these microbes and excrete excess N directly into the soil as NH₄⁺ (Bouwman and Zwart, 1994; Ferris et al., 1998; Freckman, 1988). The abundances and activities of these microbivorous nematodes may also be controlled by predatory nematodes and other fauna indirectly influencing N mineralization (Neher, 2001; Wardle and Yeates, 1993). Despite their abundance, diversity and the complex multitrophic interactions between nematodes and other soil biota, most of the experiments conducted so far have been limited to simplified experiments in which few selected species of nematodes and microbes are often inoculated in completely sterilized soil or inert media in the absence of plants (Anderson et al., 1981; Chen and Ferris, 1999; Ferris et al., 1998).

The problems of such simplified experiments dealing with a specific feeding group at a time lie not only with the difficulties of extrapolating the results to the actual field conditions but also in that part of the actual interactions are omitted, resulting in a possibly less accurate estimation of their contribution. For example, Tu et al. (2003) has estimated the contribution of plant-feeding nematodes to N mineralization based on only a single species of root parasitic nematode (*Rotylenchulus reniformis*) while Verschoor (2002) estimated the direct contribution of plant-feeding nematodes to be 2-5% of total mineralization based on the C: N ratios of all the extracted plant-feeding nematodes and their food sources. These findings have increased our understanding and may be considered as good evidences that plant-
feeding nematodes directly contribute to N mineralization. However, their indirect contributions were missing as microbes and their grazers were not included in these studies. Plant-feeding nematodes have been shown to contribute indirectly to N mineralization by releasing root exudates that increase the microbial growth and alter their community structure in favor of root-growing hormone producers (Cheng et al., 2011; Mao et al., 2007). This indirect contribution may be much more important than the direct contribution (Bonkowski, 2004; Verschoor, 2002), suggesting a possible under-estimation of the contribution of plant-feeding nematodes in these studies.

A considerable number of other experiments have demonstrated that the contribution of a few common species of bacterivorous and fungivorous nematodes to N mineralization in highly simplified experiments. Most of these experiments focused on a few selected species of bacterivorous (Anderson and Coleman, 1981; Ferris et al., 1998; Griffiths, 1994), or fungivorous nematodes (Chen and Ferris, 2000) and a few species of their respective preys in the absence of plants. Only a few, e.g. Ingham et al. (1985) have demonstrated the contribution of nematodes to N mineralization based on interactions between nematodes, microbes and plants although only one or two species of microbes and their respective nematode grazers were involved. The results from such experiments would also be less accurate to quantify the contribution of nematodes and do not adequately represent the actual situation as most parts of the interactions between and amongst microbes, nematodes and plants are missing. Thus, we argue that the entire nematode community should be included under more realistic conditions to better quantify their effect on N mineralization and to allow extrapolations to the field situations.

However, apart from reports based on soil food web model calculations in which input parameters were used by combining field measurements and several theoretical assumptions (Deruiter et al., 1994; Hunt et al., 1987), there are only few experimental studies in which the whole nematode community were included to determine N mineralization (Buchan et al., 2012; Xiao et al., 2010) even in the absence of plants. To the best of our knowledge there are no data on the contribution of the entire free-living nematode community to N mineralization
in experimental setups which include all nematode functional groups, plants and native microflora, leaving an important gap in our understanding of this topic.

To address this, we set up a microcosm incubation experiment in realistic conditions by applying gamma irradiation with a dose proven to kill nematodes and other fauna completely without significantly altering the native microbial community (Buchan et al., 2012) instead of the classical complete sterilization by autoclaving (Anderson and Coleman, 1982; Anderson et al., 1981; Ferris et al., 1998; Ingham et al., 1985; Xiao et al., 2010). Here, we also extracted and reinoculated the entire nematode community instead of a few selected species (Anderson et al., 1981; Chen and Vincx, 1999; Chen and Ferris, 1999; Ferris et al., 1998; Griffiths, 1994; Ingham et al., 1985) and in the presence of plants.

In this study, we test the hypothesis that the interactions between microbes, the entire nematode community and plants enhance the contribution of nematodes to N mineralization and plant N uptake as compared to bare soils where microbial growth and activity is limited by the absence of carbon released from roots.

4.2 Materials and Methods

4.2.1 Sample collection

Composite soil samples were randomly collected in a zigzag pattern to 15 cm depth in May 2011 from an agriculture trial field (ILVO, Merelbeke, Belgium) under organic management that had been sown with a grass-clover mixture in the previous summer. The texture was a sandy loam with a bulk density of 1.42 g cm^{-3}. At the time of sampling, the soil contained 1.06% organic carbon, 0.08% total N, 7.98 mg kg^{-1} dry soil total mineral N and had a pH (KCl) of 5.6. The fresh soil was sieved through a 5 mm mesh to remove stones and root fragments before gently homogenized. Part of it was used for nematode extraction and gamma irradiation, and the rest was stored in a cold room (+4 °C) for about three days.

4.2.2 Gamma irradiation, nematode extraction and planting

We used gamma irradiation to defaunate the soil by selectively killing all nematodes and other soil fauna without significantly altering the microbial biomass and community structure, following the procedure by Buchan et al. (2012). Briefly, part of the homogenized soil was
filled up in PVC tubes (each containing about 2.5 kg) and subjected to gamma irradiation at a
dose of 5 kGy (Sterigenics industrial facility, Fleurus, Belgium), a dose sufficient to defaunate
the soil while keeping the soil microflora largely intact (Buchan et al., 2012). Here, a 5 kGy
dose was used instead of the recommended 3 kGy at 80% WFPS in Chapter 2. The only reason
for this was the fact that this experiment was conducted before adapting the gamma
irradiation had been made. Free-living nematodes were extracted from part of the remaining
(i.e. non-irradiated soil) using an automated zonal centrifuge, a method which separates free-
living nematodes from mineral and most organic soil constituents based on their specific
density (Hendrickx, 1995). The remaining non-irradiated homogenized soil was kept in the
cold room for later use as a control.

A total of 108 experimental units were prepared by filling up PVC cores (height = 7.5 cm and
diameter = 7.5 cm) with 300 g of respective soil (36 cores with control soil and 72 cores with
irradiated soil) and gently compacted to the same bulk density as measured in the field. Half
of the irradiated samples (n=36) were reinoculated with concentrated nematodes
suspicions extracted from the same amount of soil (i.e. from 300 g soil) by spraying and
gently mixing. Forty eight cores were each sown with 15 seeds of perennial rye grass (Lolium
perenne) and reduced to 12 seedlings after germination.

4.2.3 N mineralization

N mineralization in the soil samples was monitored throughout the incubation period by
measuring mineral nitrogen (NH$_4^+$ and NO$_3^-$). Both NH$_4^+$ and NO$_3^-$ were measured from the
same aliquot colorimetrically in a continuous flow auto analyzer (Chem-lab 4, Skalar 223
Analytical, Breda, The Netherlands), following the extraction of 30 g moist soil with 60 ml 1M
KCl (1:2 ratio) after shaking for one hour. The same procedure was followed for soil samples
in planted cores. Because mineral N was almost entirely taken up by the grass, N
mineralization in planted cores was calculated as the difference between mineral N at the
start of incubation and mineral N and total N in the plant biomass at the end of incubation.
4.2.4 Microbial biomass carbon and community structure

Microbial biomass carbon ($C_{\text{mic}}$) was determined using 30 g of fresh soil by the fumigation-extraction method according to Vance et al. (1987), based on the differences in total organic carbon subtracted between the fumigated and non-fumigated samples. The extraction efficiency coefficient used for $C_{\text{mic}}$ calculations was 0.45 (Joergensen, 1996).

Changes in the structure of the microbial community were determined based on microbial membrane phospholipid fatty acids (PLFA) following a procedure explained in details by Moeskops et al. (2010) and Buchan et al. (2012). Briefly, 4 g of freeze-dried soil from each sample was sieved (2 mm) in order to homogenize and remove the root fragments and stones. This freeze-dried soil was subjected to different extraction procedures for three days. On the first day, total lipids were separated from other soil components using a multi-phase extraction mixture ($\text{CHCl}_3$, $\text{MeOH}$ and $\text{P}$ buffer) in a separatory funnel. On the second day, the extracted lipids were fractionated into three lipid classes using Solid Phase Extraction (SPE) cartridges, after which only phospholipids were kept for further analysis. The separated PLFAs were transformed into fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. After drying the FAMEs under $\text{N}_2$ gas, the FAMEs were re-dissolved in hexane containing nonadecanoic acid methyl ester (C19:0) as an internal standard FAME. Finally, individual FAMEs were identified and quantified by Gas Chromatography-Mass Spectrometry (GC-MS) on a Thermo Focus GC combined with a Thermo DSQ quadrupole MS (Interscience BVBA, Louvain-la-Neuve, Belgium) in electron ionization mode. The sums of marker fatty acid concentrations for selected microbial groups were calculated as in Moeskops et al. (2010).

For Gram-positive bacteria the sum of i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0; for Gram-negative bacteria cy17:0 and cy19:0; for the actinomycetes the sum of 10Me16:0 and 10Me18:0; for saprotrophic fungi 18:2ω6c and for arbuscular mycorrhizal fungi (AMF) 16:1ω5c. For the total bacterial community, in addition to the sum of, the marker PLFAs for Gram-positive and Gram-negative bacteria, fatty acids 15:0, 17:0 and cy19:0 were also considered. The bacterial: fungal ratio was calculated as the sum of total bacterial community divided by the fungal fatty acid 18:2ω6c.
4.2.5 Nematode extraction and identification

Throughout the incubation, all free-living nematodes were extracted from 50 g moist soil (CTR and +Nem samples) as explained above using the zonal centrifuge machine. In previous experiments Buchan et al. (2012) observed that dead nematodes in irradiated samples are extracted together with viable nematodes. Here, to separate the dead nematodes in +Nem samples, each nematode suspension was poured onto a sieve covered with a filter paper for 48 hours so that only viable nematodes migrated towards the water and collected in the plate. All viable nematodes that passed through the filter were then concentrated and counted using a dissecting microscope (40x) and were subjected to mass fixation. Counting and mass fixation were carried out at each sampling period, but the identification was done only for the first (7th day) and last sampling (86th day of the incubation). Mass slides were made in three replicates from each control and +Nem treatments. At least 250 nematodes were identified from each slide to family or when possible to genus level based on Bongers (1994) and assigned to the corresponding functional groups according to Yeates et al. (1993a). However, Filenchus was considered as fungivorous as suggested by different authors (Buchan et al., 2012; Christensen et al., 2007; Okada et al., 2002) and the remaining genera of the Tylenchidae family considered as root feeders.

4.2.6 Plant samples preparation and analysis

All the grasses planted in each core were carefully removed from the soil together with the root by gently shaking the roots to detach the soil. The above ground biomass was then briefly rinsed in distilled water to remove the dust and other contaminants. The roots were further rinsed in distilled water for an extra few minutes to make sure that soil particles were completely detached. The plant samples were then dried in an oven for 48 hours at 60 °C and grinded down to a mean particle size of 0.01 mm. Total dry biomass was calculated as the sum of the weights of both root and shoot after drying but before grinding. Total organic nitrogen was determined by Elemental analysis (Variomax CNS analyzer, Elementar, Germany) in the roots and shoots separately.
4.2.7 Statistical analysis

The experiment followed factorial design with three fixed factors: 1) treatment with three levels: CTR, +Nem and -Nem; 2) time with six levels: 0, 7, 30, 45, 65, and 86 incubation days; 3) planting with two levels: bare and planted with rye grass. By applying an analysis of variance (ANOVA) with three factors, the effects of each main factor, and all possible statistical interactions between factors could be investigated simultaneously. However, due to the presence of significant statistical interactions (p<0.05) between the three factors for most of the variables that complicate the interpretations, we split the data file based on planting and applied an ANOVA model with two fixed factors (treatment and time) for bare and planted cores separately. The assumptions of ANOVA (both normality and homoscedacity) were checked for all variables (NH$_4^+$-N, NO$_3^-$-N, total mineral N, N uptake, C$_{mic}$, dry plant biomass, total and biomarker PLFAs, and nematode feeding groups) before running ANOVA. Although only NH$_4^+$ data did not meet the assumptions, and needed log transformation, NO$_3^-$ and total mineral N was also log transformed to avoid error as they are interrelated.

Whenever the interaction term (treatment*time) was significant (p<0.05), the interpretation of ANOVA was first made based on the main fixed factors. For example, the effects of nematodes compared irrespective of incubation time before proceeding to run one way ANOVA for each time-treatment combination. Fishers’ LSD was used for mean comparisons of the main factors as it is the most powerful to detect true differences (Montgomery, 2001) while Tukey was used for the post hoc mean comparisons of the variables following significant treatment*time interactions. Mean comparisons were done mainly between -Nem and +Nem treatments both in bare and planted cores, as, the objective of this experiment was to compare the contribution of nematodes to N mineralization in the absence and presence of plant roots. Relationships among C$_{mic}$, PLFA biomarkers and nematode feeding groups were analyzed using Pearson’s pairwise correlation coefficient. All the above statistical analysis was done with SPSS version 20. The PLFA data was further analyzed by principal component analysis (PCA) using the correlation matrix in R software version 15 and mean scores were presented in a graph with error bars.
4.3 Results

4.3.1 Nematode community and dynamics

Results from the first sampling date (7th day of incubation) were considered as initial total nematode abundances and compositions in both bare and planted cores as no roots had developed yet. The efficiency of reinoculation, that is, the total nematode abundance in reinoculated samples (+Nem) at the beginning of the experiment was 69% of the total nematode abundance in the control samples. Total nematode abundance increased over time regardless of the treatments both in bare (increase of 5.1 individuals g⁻¹ soil; p = 0.06) and in planted cores (increase of 21.8 individuals g⁻¹ soil; p<0.001). More nematodes were found in CTR cores than in +Nem cores both in bare (12.2 more individuals g⁻¹ soil; p<0.01) and planted microcosms (3.0 individuals g⁻¹ soil; p>0.05).

In the beginning of the experiment, the nematode composition was dominated by bacterivores (ca 60% in CTR and 50% in +Nem cores) (Table 4.1, Figure 4.1). This was still the case at the end of the experiment in bare microcosms (ca 65% in CTR and 70% in +Nem). However, in planted microcosms, herbivores strongly dominated (ca 70% in +Nem cores) while the proportion of bacterivores decreased to less than 20% in +Nem cores.

![Figure 4.1: Relative proportion of nematode composition based on trophic groups. The first sampling is considered as an initial composition for both bare and planted soil.](image)

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Table 4.1 Mean abundances (and standard error of the mean) for dominant families, main trophic groups and total free-living nematodes (individuals g⁻¹ dry soil) at the beginning and end of incubation period. The p values are based on two way ANOVA model run separately for bare and planted soils.

| Factors     | Tylenchidae | Dolichodoridae | Paratylenchidae | Others | Herbivores | sub total | Filenchus | Aphelenchidae | Aphelenchoidae | sub total | Dauer | Rhabditidae | Cephalobidae | Others | Bacteri- | sub total | Omni- | Total |
|-------------|-------------|----------------|-----------------|--------|------------|-----------|-----------|--------------|---------------|-----------|-------|-------------|-------------|--------|vores    |------------|vores |-------|
| Bare microcosms |             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| Control     | 7           | 2.35(0.11)     | 1.50(0.24)      | 1.49(0.51)| 1.11(0.22)| 6.45(0.67)| 1.33(0.32)| 0.98(0.22)   | 1.43(0.63)    | 3.75(0.60)| 3.13(0.63)| 3.60(0.36)  | 8.40(1.61)  | 1.95(0.60)| 17.08(2.50) | 0.23(0.05) | 27.51  |
|             | 86          | 2.57(0.10)     | 1.85(0.53)      | 0.55(0.06)| 0.79(0.23)| 5.75(0.47)| 1.07(0.30)| 1.45(0.79)   | 3.68(1.29)    | 6.19(0.54)| 1.37(0.48)| 6.62(1.42)  | 14.93(2.10) | 0.47(0.20)| 23.40(2.52) | 0.90(0.21) | 36.24  |
| PlusNems    | 7           | 3.57(0.76)     | 1.67(0.46)      | 1.20(0.23)| 1.14(0.23)| 7.58(0.61)| 1.01(0.26)| 0.37(0.04)   | 1.05(0.29)    | 2.43(0.55)| 0.73(0.34)| 3.18(0.68)  | 4.33(0.61)  | 0.57(0.09)| 8.81(1.60)  | 0.10(0.10) | 18.92  |
|             | 86          | 1.47(0.62)     | 0.98(0.48)      | 0.46(0.27)| 0.32(0.08)| 3.22(1.33)| 0.72(0.44)| 0.56(0.12)   | 0.95(0.43)    | 2.23(0.97)| 0.34(0.14)| 8.65(1.75)  | 5.41(1.78)  | 0.40(0.11)| 14.80(1.94) | 0.22(0.13) | 20.47  |
|             |             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (time)    |             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.05           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (treatment)|             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.16           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (time*treatment)|         |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.18           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| Planted microcosms |         |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| Control     | 7           | 2.35(0.11)     | 1.50(0.24)      | 1.49(0.51)| 1.11(0.22)| 6.45(0.67)| 1.33(0.32)| 0.98(0.22)   | 1.43(0.63)    | 3.75(0.60)| 3.13(0.63)| 3.60(0.36)  | 8.40(1.61)  | 1.95(0.60)| 17.08(2.50) | 0.23(0.05) | 27.51  |
|             | 86          | 4.82(0.87)     | 11.59(0.88)     | 2.04(0.82)| 1.23(0.21)| 19.69(1.12)| 2.61(0.72)| 1.24(0.26)   | 2.05(0.83)    | 5.90(0.88)| 1.44(0.43)| 5.71(0.76)  | 9.46(0.86)  | 0.63(0.44)| 17.24(1.74) | 0.85(0.32) | 43.68  |
| PlusNems    | 7           | 3.57(0.76)     | 1.67(0.46)      | 1.20(0.23)| 1.14(0.23)| 7.58(0.61)| 1.01(0.26)| 0.37(0.04)   | 1.05(0.29)    | 2.43(0.55)| 0.73(0.34)| 3.18(0.68)  | 4.33(0.61)  | 0.57(0.09)| 8.81(1.60)  | 0.10(0.10) | 18.92  |
|             | 86          | 9.58(2.08)     | 17.47(0.69)     | 3.97(1.65)| 0.69(0.22)| 31.7(2.87)| 2.12(0.26)| 1.17(0.16)   | 2.01(0.48)    | 5.29(0.82)| 0.06(0.06)| 3.63(0.94)  | 4.30(1.04)  | 0.73(0.19)| 8.71(1.76)  | 0.54(0.07) | 46.24  |
|             |             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (time)    |             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.00           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (treatment)|             |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.00           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
| p (time*treatment)|         |                |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
|             |              | 0.06           |                 |        |            |           |           |              |               |           |       |             |             |        |         |            |       |       |
4.3.2 Microbial biomass and community structure

The effects of nematodes on $C_{\text{mic}}$ varied over time in both bare and planted microcosms (Figure 4.2). In bare microcosms, the average concentration of $C_{\text{mic}}$ over time in +Nem cores was lower than in -Nem cores (mean difference = 4.3 mg kg$^{-1}$ dry soil; $p = 0.36$). In the presence of plants, +Nem cores showed significantly lower $C_{\text{mic}}$ than -Nem cores (mean difference = 53.8 mg kg$^{-1}$ soil; $p<0.001$) at the end of the incubation. The average $C_{\text{mic}}$ over time was also lower in +Nem cores than in -Nem cores (mean difference of 6.8 mg kg$^{-1}$ dry soil) but statistically not significant ($p = 0.11$).

The total PLFAs and PLFAs in each microbial group (Gram positive and Gram negative bacteria, Arbuscular mycorrhizal fungi (AMF) and saprophytic fungi and actinomycetes) significantly ($p<0.01$) decreased over time both in bare and planted soils (Table 4.2). CTR cores showed significantly ($p<0.01$) higher PLFA concentrations than +Nem and –Nem cores regardless of the incubation time in both bare and planted soils. However, no significant differences were observed between +Nem and –Nem cores. The B:F ratio was not significantly affected by both time and treatment factors ($p>0.05$) in bare soils; however, it was significantly ($p<0.01$) reduced over time in planted microcosms.

Figure 4.2 Mean microbial biomass carbon, $C_{\text{mic}}$ ($n=4$ with standard error of the mean) in bare (a) and in planted (b) soils.
Figure 4.3. Principal component scores of PLFA in bare soils (a) and in planted soils (b) in control (CTR), no nematodes (-Nem) and with nematodes (+Nem). Numbers after the treatment codes indicate 7, 30 and 86 days of incubation. For each treatment combination given, points and error bars represent the mean scores (n=4) and standard error of the mean respectively.
Table 4.2 Mean concentrations of total PLFA and selected biomarkers for major microbial groups in nmole g⁻¹ dry soil with standard error of the mean in parenthesis. The p values are based on two way ANOVA model (ns refers to p values >0.05).

<table>
<thead>
<tr>
<th></th>
<th>Total PLFA</th>
<th>Gram Positive</th>
<th>Gram Negative</th>
<th>Actinomycets</th>
<th>AMF</th>
<th>Saprophytic Fungi</th>
<th>BF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bare microcosms</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>7 control</td>
<td>27.23 (0.24)</td>
<td>4.90 (0.05)</td>
<td>2.97 (0.03)</td>
<td>2.19 (0.02)</td>
<td>1.40 (0.01)</td>
<td>1.17 (0.02)</td>
<td>7.17 (0.13)</td>
</tr>
<tr>
<td>PlusNems</td>
<td>20.79 (0.53)</td>
<td>3.69 (0.10)</td>
<td>1.90 (0.08)</td>
<td>1.37 (0.03)</td>
<td>0.85 (0.02)</td>
<td>0.71 (0.06)</td>
<td>8.65 (0.67)</td>
</tr>
<tr>
<td>MinusNems</td>
<td>22.37 (0.54)</td>
<td>4.07 (0.15)</td>
<td>2.17 (0.10)</td>
<td>1.48 (0.05)</td>
<td>0.87 (0.01)</td>
<td>0.72 (0.05)</td>
<td>9.42 (0.74)</td>
</tr>
<tr>
<td>86 control</td>
<td>19.08 (0.73)</td>
<td>3.69 (0.17)</td>
<td>1.98 (0.07)</td>
<td>1.45 (0.05)</td>
<td>0.67 (0.01)</td>
<td>0.93 (0.06)</td>
<td>9.04 (0.37)</td>
</tr>
<tr>
<td>PlusNems</td>
<td>14.30 (0.55)</td>
<td>2.63 (0.16)</td>
<td>1.20 (0.06)</td>
<td>0.85 (0.05)</td>
<td>0.55 (0.04)</td>
<td>0.5 (0.04)</td>
<td>7.69 (0.79)</td>
</tr>
<tr>
<td>MinusNems</td>
<td>13.27 (1.19)</td>
<td>2.51 (0.32)</td>
<td>1.28 (0.17)</td>
<td>0.76 (0.07)</td>
<td>0.46 (0.03)</td>
<td>0.51 (0.03)</td>
<td>9.13 (1.24)</td>
</tr>
<tr>
<td>p(time)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.73</td>
</tr>
<tr>
<td>p(treatment)</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.26</td>
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<tr>
<td>p(time*treatment)</td>
<td>ns</td>
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<td>ns</td>
<td>ns</td>
<td>0.00</td>
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<td>ns</td>
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<tr>
<td><strong>Planted microcosms</strong></td>
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<tr>
<td>7 control</td>
<td>27.23 (0.24)</td>
<td>4.90 (0.05)</td>
<td>2.97 (0.03)</td>
<td>2.19 (0.02)</td>
<td>1.40 (0.01)</td>
<td>1.17 (0.02)</td>
<td>7.17 (0.13)</td>
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<tr>
<td>PlusNems</td>
<td>20.79 (0.53)</td>
<td>3.69 (0.10)</td>
<td>1.90 (0.08)</td>
<td>1.37 (0.03)</td>
<td>0.85 (0.02)</td>
<td>0.71 (0.06)</td>
<td>8.65 (0.67)</td>
</tr>
<tr>
<td>MinusNems</td>
<td>22.37 (0.54)</td>
<td>4.07 (0.15)</td>
<td>2.17 (0.10)</td>
<td>1.48 (0.05)</td>
<td>0.87 (0.01)</td>
<td>0.72 (0.05)</td>
<td>9.42 (0.74)</td>
</tr>
<tr>
<td>86 control</td>
<td>19.08 (0.73)</td>
<td>3.69 (0.17)</td>
<td>1.98 (0.07)</td>
<td>1.45 (0.05)</td>
<td>0.67 (0.01)</td>
<td>0.93 (0.06)</td>
<td>9.04 (0.37)</td>
</tr>
<tr>
<td>PlusNems</td>
<td>14.30 (0.55)</td>
<td>2.63 (0.16)</td>
<td>1.20 (0.06)</td>
<td>0.85 (0.05)</td>
<td>0.55 (0.04)</td>
<td>0.5 (0.04)</td>
<td>7.69 (0.79)</td>
</tr>
<tr>
<td>MinusNems</td>
<td>13.27 (1.19)</td>
<td>2.51 (0.32)</td>
<td>1.28 (0.17)</td>
<td>0.76 (0.07)</td>
<td>0.46 (0.03)</td>
<td>0.51 (0.03)</td>
<td>9.13 (1.24)</td>
</tr>
<tr>
<td>p(time)</td>
<td>0.02</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>p(treatment)</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.21</td>
</tr>
<tr>
<td>p(time*treatment)</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.00</td>
<td>ns</td>
<td>0.01</td>
</tr>
</tbody>
</table>
In bare soils the first principal component (PC1), which explained 77% of the total variation, mainly separated samples based on the factor time (t1 and t2 on one side and t5 on the other side) (Figure 4.3a). PC2 mainly separated all CTR samples from +Nem and -Nem samples and it also separated +Nem and –Nem samples at the end of the incubation. In planted soils, PC1 explained 63% of the total variation and fairly separated the CTR from +Nem and –Nem samples after 30 and 86 incubation days (Figure 4.3b). PC2 (18% variation) mainly separated the control from +Nem and- Nem samples. Each of the PCs was uniformly loaded by all individual FAME values (Appendix 3).

A statistically significant positive correlation was observed between total PLFA and $C_{\text{mic}}$ at the start ($r = 0.87$, p<0.01) and end of the incubation both in bare ($r = 0.90$, p<0.01) and planted microcosms ($r = 0.79$, p<0.01). Fungivorous nematodes showed a positive correlation between saprophytic fungi and AMF biomarkers ($r = 0.52$; $p = 0.15$ for fungi and $r = 0.74$; $p = 0.05$ for AMF) at the start of the experiment. However, at the end of incubation, negative correlation was observed in planted soils between the above fungi biomarkers and fungivores ($r = -0.34$ and -0.18 for saprophytic fungi and AMF respectively; $p>0.05$ for both), while a stronger positive correlation was observed in bare soils ($r = 0.93$ and 0.77 for saprophytic fungi and AMF respectively, p<0.05). Gram-positive and Gram-negative biomarkers showed a positive correlation with bacterivorous nematodes at the start of the incubation ($r = 0.88$ and 0.83 for Gram-positive and Gram-negative respectively; p<0.05 for both) and at the end of incubation both in bare ($r = 0.54$ and 0.73) and planted microcosms ($r = 0.87$ and 0.93).

4.3.3 Soil mineral N dynamics and N mineralization

Bare soil

$\text{NH}_4^+$-N concentration in CTR cores was significantly lower (p<0.001) than in both +Nem and –Nem cores. $\text{NH}_4^+$-N increased in +Nem cores reaching a peak value within 30 days of incubation and consistently decreased throughout the remaining incubation period while it increased slightly in –Nem cores (Figure 4.4a). However, significantly lower $\text{NH}_4^+$-N was observed in +Nem cores than –Nem cores only after 65 days of incubation (mean difference 17.7 mg $\text{NH}_4^+$-N kg$^{-2}$ dry soil, p<0.05).
The concentration of NO$_3^-$-N was also consistently higher in +Nem cores than in -Nem cores throughout the experiment, and significantly higher after 30 days of incubation (mean differences 9.3, 11.6, and 26.6 mg NO$_3^-$-N kg$^{-1}$ dry soil after 45, 65, and 86 days respectively; p<0.05) as shown in Figure 4.4b. NO$_3^-$-N in CTR cores also increased consistently over time but the concentration was significantly higher than in –Nem after 86 days of incubation only. Similarly total mineral N (NH$_4^+$-N + NO$_3^-$-N) was higher (5.1 mg N kg$^{-1}$ soil on average) in +Nem cores throughout the incubation period than in –Nem cores (Figure 4.4c). A statistically significant difference was observed only after 45 incubation days (mean difference = 7.9 mg N kg$^{-1}$ dry soil; p<0.05). Total mineral N in the CTR cores also increased throughout the incubation but the concentration was significantly lower than in both other treatments. The net mineralization calculated as the difference in total mineral N at the beginning (0 days) and end of the incubation (86 days) was 36.6 and 27.6 mg N kg$^{-1}$ dry soil in +Nem and -Nem cores respectively, that is, about 32% higher in +Nem cores.

**Figure 4.4.** Total N mineralization and N dynamics in bare (a, b and c) and planted (d) soils (mean values and standard error of the mean). In planted cores total mineral N is calculated as the sum of mineral N in the soil and N in the plant roots and shoots.
Planted soils

Mineral N in planted cores reached undetectable levels particularly towards the end of the experiment due to the N uptake by the grass. In planted cores N taken up by plants was taken into account, that is, total mineral N at each time refers to the sum of mineral N in the soil and N taken up by the grass. No significant differences (p<0.05) were found between +Nem and -Nem cores at each sampling date, but CTR cores had significantly (p<0.05) lower total mineral N than both treatments (Figure 4.4d). The net mineralization in +Nem and -Nem cores was 28.2 and 30.6 mg N kg\(^{-1}\) dry soil respectively, or about an 8% decrease in planted +Nem cores.

4.3.4 N uptake and plant dry biomass

The effects of nematodes on plant dry biomass production and N uptake changed over time as it was the case for N mineralization (p<0.03 for N uptake; p<0.001 for dry biomass). Plant dry biomass (shoots and roots) was consistently higher in +Nem cores until 65 days of incubation and no difference was observed with -Nem cores afterwards (Figure 4.5). The CTR cores showed significantly lower (p<0.05) dry biomass than in both treatments. N uptake was higher in +Nem cores within about 60 days of incubation and was consistently lower than in -Nem cores afterwards, although the difference was not statistically significant (p>0.05) (Figure 4.5). N uptake in CTR followed more or less the same trend over time but was significantly (p<0.05) lower than both treatments.

Figure 4.5. N uptake and total dry biomass (shoots and roots) in mg per pot (mean values, n=4 and standard error of the mean).
4.4 Discussion

The aim of this experiment was investigating the effects of the whole nematode community on nitrogen mineralization in a complex soil food web in which native microbes, the entire free-living nematode community and plants interact. Nematode reinoculation was successful both in terms of abundance (about 70% efficiency) and composition as all the feeding groups which were found in CTR were also found in +Nem samples. As expected, CTR and +Nem samples differed in some biological and chemical parameters such as $C_{mic}$ and total mineral $N$ at the start and throughout the incubation period. This is apparently because the CTR samples contained the full biotic community (the complete soil food web), whereas +Nem samples contained no other fauna than nematodes and because gamma irradiation caused a nutrient flush which resulted in higher mineral nutrient concentrations in +Nem samples than in CTR samples. Therefore, the effects of nematodes on N mineralization and microbial properties were determined by comparing +Nem with –Nem samples as both have similar biological and chemical properties except the presence or absence of nematodes.

4.4.1 Nematode community and dynamics

The total nematode abundances increased over time both in bare and planted cores, and in line with our expectation the increase in planted soil was threefold and highly significant compared to the bare soils. Griffiths (1990b) has also found a 27% increase in nematode abundance in the rhizosphere as compared to the surrounding soil.

At the beginning of the incubation, bacteria feeding nematodes and plant feeders shared the largest proportion of total nematodes which is comparable to a reported functional groups proportion in agro ecosystems in general (Bardgett et al., 1999a; Neher and Powers, 2004). However, at the end of incubation the composition was significantly different in bare (dominated by bacterivores) and planted soils (dominated by herbivores) indicating the presence of plants not only affects total nematode abundance but also their community structure. Several authors have reported that the presence, abundance and identity of plants change both the microbial and nematode community compositions (De Deyn et al., 2004; Eisenhauer et al., 2011; Zak et al., 2003). In this study also planting with only one species of
ryegrass may have influenced the composition over time to be dominated by phytophagous (herbivores) nematodes (Wasilewska, 1995). Moreover, given that plants are food sources for herbivores, it is logical that their abundance increases in the presence of plants and decreases in their absence. However, about half of the plant feeders survived after 86 days of incubation in bare soils suggesting that they might be employing additional feeding strategies, such as feeding facultatively on fungi using their stylet (Okada et al., 2002).

In contrast to the hypothesis that the carbon released from root exudates stimulates microbial growth and thus microbivorous nematodes (Bardgett et al., 1999a), the interactions between plants and the entire free-living nematode community did not show significant effects particularly on the abundance of bacterivores. This could partly be explained by the limited capacity of rye grass to release sufficient root exudates which significantly affect the abundance and activity of bacterivores. Previous studies indicated that the abundance of bacterivores and omnivores significantly depend on the plant species (Bezemer et al., 2010) and particularly the presence of grasses reduced the bacterivores by up to 37%, but no explanation was given (Eisenhauer et al., 2011). Bacterivores significantly increased only in bare soils over time in this experiment while fungivorous and omnivorous nematodes significantly increased both in bare and planted cores over time.

4.4.2 Microbial biomass and community structure

Nematodes showed contrasting effects on \( C_{\text{mic}} \) under different simplified experimental set ups in which the microflora were reinoculated (Bardgett et al., 1998; Mikola and Setala, 1998). Reinoculating the microflora in sterilized soil could cause experimental errors not only because of the difficulties to reconstruct the native microflora both in terms of abundances and compositions, but also because of the easier accessibility of the reinoculated microflora for potential overgrazing by nematodes (Djigal et al., 2004; Xiao et al., 2010). Such potential problems have been minimized in our experiment as we avoided reinoculation but applied instead a low dose gamma irradiation that keeps the native microflora largely intact. The entire free-living nematode community did not significantly change \( C_{\text{mic}} \) in bare soils, but we found higher \( C_{\text{mic}} \) in the presence of plants than in bare soils, which is likely due to the extra
carbon released from the roots into the soil as herbivores are feeding on the roots (Alphei et al., 1996).

Previous studies have demonstrated that the interaction between nematodes and plant roots increases $C_{mic}$ as more root exudates are released into the soil following root infestations by selected obligatory plant feeding nematodes (Bardgett et al., 1999b; Yeates et al., 1999a). However, in contrast to these findings, the presence of an entire nematode community did not significantly change $C_{mic}$ in our experiment, although $C_{mic}$ was higher in +Nem samples during most of the incubation period than in –Nem (Figure 4.2). A possible explanation for this could be that there was less root infestation by herbivorous nematodes, which consequently resulted in the release of less root exudates and which did not cause a significant increase of $C_{mic}$. The positive feedbacks for the interaction between nematodes and roots on $C_{mic}$ from previous reports were mainly based on only a selected species of *Heterodera* and *Meloidogyne*, sedentary nematodes which are identified to cause high root damage and consequently release large amounts of root exudates (Denton et al., 1999; Van Gundy et al., 1977; Yeates et al., 1999a). In this study, the nematode community was actually dominated by Dolichodoridae, ectoparasitic nematodes whose effect on root damage might be lower as they feed only by inserting their stylet while their body part remains outside the root (Decraemer and Geraert, 2006).

Statistically significant positive relationship between total PLFA concentrations and $C_{mic}$ supports previous findings on several soil types (Bailey et al., 2002). Although the PCs explained more than 80% of the total variation both in bare and planted cores, each FAME was loaded on the PCs more or less uniformly. This shows that no single microbial group was dominating, and inoculating the entire nematode community did not alter the microbial community structure both in bare and in planted cores. The presence of nematodes also did not significantly change the biomass of each microbial group both in bare and planted cores, confirming the results of the PCA. Previous studies have indicated that bacterivorous nematodes change the microbial community, particularly the bacterial community (Djigal et al., 2010; Djigal et al., 2004; Griffiths et al., 1999; Mao et al., 2007; Xiao et al., 2010). Although bacterivores dominated in bare soils towards the end of the incubation, no significant difference was observed in the major groups of bacterial community (G+ and G-). The absence
of significant differences in any of the microbial functional groups between +Nem and –Nem in this experiment suggests that the effects of nematodes on microbial community structure could depend on nematode identity and multitrophic interactions. It could be possible that the individual effects of the reported nematodes species in the above mentioned simplified experiments could not be observed here as a result of multitrophic interactions in this experiment.

A significant positive correlation between bacterial feeding nematodes and both gram positive and gram negative bacteria in bare and planted cores supports previous findings that bacterivorous nematodes could increase the abundance of bacteria. This could be by transporting them to resource-rich locations by mechanisms such as adhering to their cuticle and defecating the ingested bacteria in a viable condition (Freckman, 1988). The positive correlation between fungivorous nematodes and fungi (both saprophytic and AMF) in bare cores also suggests the presence of such transporting mechanisms (Bird, 1987). However, in agreement with Yeates et al. (1997), the correlation was not significant in planted cores suggesting that fungivores might be feeding on roots using their stylet and may not depend on the fungal biomass. In this experiment we did not attempt to investigate whether different fungivores feed alternatively on plant roots or not. Nevertheless, based on previous studies which considered some of the fungivorous nematodes, particularly *Filenchus* as plant feeders (Ruess, 1995) or as plant and root feeders (Hanel, 2000), we think that fungivores may alternatively have been feeding on plant roots.

4.4.3 Nitrogen dynamics and mineralization

The presence of the entire nematode community increased nitrification significantly in bare soils. Previous studies have demonstrated that the presence of single species of bacterivorous nematode or the presence of nematode community that were intentionally reared to be dominated by bacterivores increase nitrification (Bouwman et al., 1994; Bouwman et al., 1993; Xiao et al., 2010). Here, we provide additional evidence showing that inoculation of an entire nematode community and the resulting interactions within and between nematodes and microbes also increase nitrification in bare soils. Recently, Xiao et al. (2010) have shown that the interactions between nematodes and microbes increase nitrifying bacterial
community, particularly nitrosomonas. Unfortunately, the presence of these ammonia oxidizing communities and their dynamics was not monitored in this experiment. The observed significant decrease in NH$_4^+$-N (the substrate for nitrification) in the presence of nematodes in this experiment could be attributed to the increased nitrification.

In bare soils, total mineral nitrogen significantly increased in the presence of nematodes supporting previous findings using simplified experimental conditions. The net increase of mineral N in the presence of nematodes was about 32% which is higher than previous estimations of 8-19% of total N mineralization by bacterivores and predators under field conditions (Beare, 1997; Neher, 2001) and comparable with that of 30% by microbivorous nematodes and protozoa (Bouwman and Zwart, 1994; Verhoef and Brussaard, 1990). Several responsible mechanisms for this enhanced process have been reported (Chen and Ferris, 2000; Deruiter et al., 1993; Freckman, 1988; Hunt et al., 1987; Ingham et al., 1985) such as the release of excess NH$_4^+$-N as a result of relatively low assimilation and production efficiency of nematodes and/or their higher C:N ratio than their prey. The increase in total mineral N in bare soils in this experiment could be due to the combination of these mechanisms.

In contrast to our expectation we found no significant effect of nematodes on N mineralization in the presence of plants. Previous experiments that investigated the effect of nematodes on N mineralization in the presence of plants are very limited and widely differ in their findings. Ingham et al. (1985) found a significant increase in mineral N and shoot N in samples inoculated with a single species of bacteria, fungi and their grazers in comparison to the sterile control. However, Bardgett and Chan (1999) found no significant effect on N mineralization after inoculating a mixed community of nematodes which were highly dominated by bacterivores in a Montane ecosystem. In our study, the presence of the entire nematode community that became dominated by herbivores over time did not increase N mineralization and N uptake. The dry biomass also did not significantly increase in the presence of nematodes although higher biomasses were recorded in +Nems until 65 days of incubation (Figure 4.5).

The absence of this significant effect of nematodes on N mineralization could be attributed to the plant interaction effect on nematode community composition as bare soil was dominated by bacterivores while planted soil was dominated by herbivores (Figure 4.1). Bacterivorous
nematodes have been shown to increase N mineralization in several reports when inoculated alone (Anderson et al., 1981; Bouwman et al., 1994; Coleman D C et al., 1977; Ingham et al., 1985; Woods et al., 1982) or in combination with other few nematode communities (Xiao et al., 2010). But the experimental evidences for the positive effect of herbivores on N mineralization are limited and are mainly based on inoculating a few selected species of known root-feeding nematodes (Bardgett et al., 1999b; Tu et al., 2003). Although the nematode community became dominated by herbivores over the incubation time in the current study, no significant effect on N mineralization and uptake was observed. Our findings rather support the view that nematodes mainly contribute to N mineralization by grazing on microbes (Bouwman and Zwart, 1994) and that root exudation is only sufficient to allow N recycling that is lost from the plant by exudation, rather than to stimulate N mineralization from soil organic matter (Bonkowski, 2004; Griffiths and Robinson, 1992; Robinson and Heald, 1989; Robinson et al., 1989). The indirect role of herbivores by releasing more root exudates, which in turn fuels microbial populations and subsequently attracts microbial grazers, could depend on the identity of nematodes and the amount of root exudates released. The majority of the herbivores in our experiment belonged to the family Dolichodoridae which are ectoparasites (Decraemer and Geraert, 2006) that probably cause less root damage compared to endoparasites, who not only induce releasing C as exudates, but also produce enzymes that facilitate organic matter decomposition through partially degrading cellulose (Tu et al., 2003). The amounts of root exudates released into the soil, and the change in microbial community structure that favors hormone-producing bacteria and the subsequent root growth initiation were not determined in this experiment. Another explanation could be that a dissolved organic matter flush during and immediately after irradiation might be sufficient and plants may not be benefiting from the presence of grazers, i.e. bacterial mineralization activity alone probably provided enough N for the plants (Bjørnlund et al., 2012).

4.5 Conclusions

In this experiment, we have shown that the interactions within and between the entire free-living nematode community and the native microflora significantly increase nitrification, N
mineralization and change nematode composition over time in bare soil. However, further interactions with plant roots did not significantly increase N mineralization and N uptake. The initial nematode community composition changed over time, and was significantly dominated by bacterivores and herbivores in bare and planted soils respectively, which could be a possible explanation for the observed contrasting contribution of free-living nematodes to N mineralization in bare and planted soils. These results imply the importance of experimental setups in conditions that are as realistic as possible, i.e. in which the native microflora, nematodes and plants interact in order to arrive at a realistic estimation of the contribution of nematodes to N mineralization. The experimental set-up adopted here may therefore proof extremely valuable in future ecological studies on the role of soil organisms in crucial processes such as nutrient cycling. It should be noted that 5 KGy dose of gamma irradiation decreased $C_{mic}$ and increased $NH_4^+$-N concentration at the beginning of the incubation. The estimated contribution of nematodes to N mineralization in this experiment could be verified and refined with measurements of more plant related parameters such as root exudates, root growth hormones and root structures and perhaps other measurements of bacterial activity, abundance and community structure. The selective sterilization by gamma irradiation applied in this study could also be further optimized to minimize the potential side effects, such as on a nutrient flush.
Chapter 5:

The influences of the entire free-living nematode community on nutrient mineralization and plant nutrient uptake in microcosms amended with grass-clover.
Abstract

Studies which quantified the role of nematodes on nutrient cycling often focused on nitrogen (N). Data on their contribution to other nutrients such as phosphorus (P) and sulphur (S) is scarce. Reports also suggested that the contribution of nematodes to nutrient cycling often becomes significant only when the microbial mediated mineralization is not sufficient, i.e. under conditions of low nutrient availability. In this Chapter we aimed to study the contribution of nematodes to N, P and S availability and plant uptake under conditions of high nutrient availability. To do so, an incubation experiment was conducted for three months in microcosms amended with grass clover and planted with rye grass for 3 months. The contribution of nematode to N, P and S availability and plant nutrient uptake was quantified as a simple difference between two treatments with (+Nem) and without (-Nem) nematodes. N and P availability and plant N and P uptake was significantly higher in +Nem than –Nem throughout the incubation period. Sulphate availability was significantly higher in +Nem during the initial weeks of incubation, but there was no significant difference in plant S uptake. The active microbial biomass and community structure changed in the presence of plants. The abundances of nematodes significantly increased over time and was particularly dominated by herbivorous nematode. Bacterivorous nematodes significantly increased from the early stage of the incubation on. These findings were in contrast to the effects of nematodes on nutrient availability without amendment. The current findings show that the contribution of nematodes to nutrient cycling is significant under conditions of high nutrient availability.
5.1 Introduction

Several findings have consistently shown that nematodes contribute to organic matter decomposition and nutrient mineralization in microcosms without plants (Anderson et al., 1981; Chen and Ferris, 1999; Coleman et al., 1977; Xiao et al., 2010). However, reports on their contribution to nutrient mineralization in the presence of plants are scarce, and the findings are inconsistent. For example, Ingham et al. (1985) found increased N mineralization and plant N uptake after conducting an experiment with a simplified trophic assemblage using a single species of bacteria and a bacterial feeding nematode. In another experimental setup with a more complex trophic assemblage (dominated by bacterivores, but with a few fungivores and herbivores), no significant increase in N and P mineralization and plant uptake was found (Bardgett and Chan, 1999). Recently, in our own experimental work, no significant increase in N uptake and plant biomass yield was found in the presence of the entire nematode community as compared to their absence (Gebremikael et al., 2014b).

It has often been suggested that the contribution of nematodes and other soil fauna becomes significant only when the microbial mediated mineralization is not sufficient, which is the case usually under conditions of low available nutrients (Huhta, 2007; Ingham et al., 1985; Teuben, 1991). Under higher nutrient availabilities (particularly N), it has been suggested that the additional contribution of nematodes or other fauna to nutrient mineralization is insignificant as the microbes can then make sufficient nutrient available by mineralization for themselves and for the plants as well (Bjørnlund et al., 2012; Ingham et al., 1985). However, these suggestions are based on simplified experimental setups or soil food web model calculations (Osler and Sommerkorn, 2007). One of the factors that determines the N mineralization and immobilization process in the soil is the C:N ratio of the substrates, as depending on this C:N ratio, N may become available or immobilized. The interactions between the entire free-living nematode community and a range of organic amendments in microcosms without plants resulted in an increased in net N mineralized from each amendment (Gebremikael et al., 2014b). To the best of our knowledge, there are no data on the collective contribution of the entire free-living soil nematode community to nutrient mineralization and plant nutrient uptake in organically amended soils. As we already studied the effect of the entire free-living nematode community on N mineralization under nutrient limited condition (no
amendments), conducting a similar experiment under nutrient rich conditions (with amendment) provides an opportunity to compare and synthesize their effect under these two contrasting scenarios.

Nematodes not only contribute to N mineralization, but also to the availability of phosphorus and sulphur as they ingest more nutrients than required and the excess is excreted in mineral or readily mineralizable forms such as amino acids, NH$_4^+$-N and PO$_4^{3-}$ (Anderson et al., 1981; Banerjee and Chapman, 1996; Ferris et al., 2012; Ingham et al., 1985; Wang et al., 2004a; Wang et al., 2004b). Wang et al. (2004a) also reported on the contribution of nematodes to the availability of other major nutrients such as Ca, Mg and K after analysing correlations between these nutrient availabilities and nematode community densities in agricultural fields. These findings suggest that nematodes influence the availability and plant uptake of not only N but also other major plant nutrients particularly P and S. However, very little is known about the influence of nematodes on plant P and S uptake. The available data on P uptake is very limited and again often based on simplified experimental setups such as a single species of nematodes and or a single species of bacteria or using non-soil media (Irshad et al., 2011). The findings of these experimental set-ups do in no way represent the actual field conditions in which several species of nematodes and microorganisms are interacting.

In order to determine the influence of nematodes on nutrient cycling and plant uptake under high available nutrient conditions, we set up an incubation experiment with grass-clover amended microcosms in the presence of plants. In a previous study (Gebremikael et al., 2014a), clover amendment resulted in a higher abundance of microbial biomass and microbivorous nematodes compared to other amendments with a higher C:N ratio (e.g. compost) in the absence of plants. The objective of this experiment was to quantify the contribution of nematodes to the mineralization and plant uptake of N, P and S under conditions of high nutrient availability. We hypothesized that additional C and N from an organic amendment would stimulate a rapid increase in microbial biomass. Microbivorous nematodes, particularly enrichment opportunists, feeding on the increased microbial biomass may reproduce rapidly, and may excrete more nutrients. Because of the addition of
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grass-clover (C:N ratio of around 10) we hypothesized that there would be less microbial N immobilization thus leading to increased plant nutrient availability, uptake and growth.

5.2 Materials and methods

5.2.1 Sample collection and preparation

The same composite soil samples, that were used for in the C mineralization experiment (Chapter 3) were also used in this experimental set up and we refer to that Chapter for the description of the general soil properties. Part of the soil was kept separately for nematode extraction, and the remaining soil was prepared for gamma irradiation and incubation as follows.

A bulk sample of fresh grass-clover was collected from the same field and was chopped into small pieces and used as an organic amendment. Five replicates of representative chopped grass-clover subsamples were put in an oven at 60 °C until a constant weight was achieved and the total C and N contents were measured by elemental analysis (CNS Variomax, Elementar Germany). The C:N ratio of the grass clover was 10.7. An appropriate amount of fresh grass-clover was weighed and mixed with the soil in each of the PVC columns at a rate equivalent to 4.96 tons DM ha\(^{-1}\) (representing an addition equivalent to that was equivalent to 200 kg N ha\(^{-1}\)). The moisture content of the soil in each PVC column was then adjusted to 80% water filled pore space (WFPS) by adding demineralized water as explained in Chapter 3.

5.2.2 Gamma irradiation and moisture content adjustment

To defaunate the soil, gamma irradiation was applied, using the same procedures as explained in Chapter 3. All the PVC columns were subjected to a 3 kGy dose of gamma irradiation at the Synergy Health sterilizing company, Etten-Leur, The Netherlands. The exact dose applied to each PVC column was measured by a dosimeter and the average of the dosimeter readings was 3.0 kGy.

The moisture content of samples (80% WFPS at the time of irradiation) was reduced to slightly lower than 50% WFPS immediately after irradiation by spreading the soil samples at room temperature. All the samples were adjusted to a moisture content of exactly 50% WFPS by adding the appropriate amount of demineralized water immediately before the incubation
experiment was started. All free-living nematode populations were extracted from part of the remaining (i.e. non-irradiated) soil using an automated zonal centrifuge machine as explained further (5.2.4).

5.2.3 Treatments and experimental setup

The experiment had two treatments each with four replicates; 1) defaunated and reinoculated with nematodes (+Nem) and 2) defaunated and without nematodes (-Nem). A total of 48 experimental units was prepared by filling up PVC cores (height = 7.5 cm and diameter = 7.55 cm) with 400 g of amended soil and gently compacted to a bulk density of 1.4 Mg m$^{-3}$. Half of the cores (n=24) were reinoculated with a concentrated nematode suspension extracted from the same amount of soil (that is, from 400 g soil) by spraying and gently mixing as explained in Chapter 4. Each core was sown with 32 seeds of perennial rye grass (Lolium perenne) and reduced to 28 seedlings after germination. The incubation experiment was carried out for 105 days in a growth chamber with a constant temperature (17°C) and light regime (16/8 day/night regime). The moisture content in each sample was kept constant at 50% WFPS by adding demineralized water as needed, once a day at the beginning of the experiment and twice a day as evapotranspiration increased following the rapid growth of the grass. Four replicates of each treatment were removed and destructively sampled after 7, 24, 47, 67, and 105 days of incubation for chemical and biological analyses.

5.2.1 Nematode extraction, identification and analysis of community indices

Throughout the incubation, all free-living nematodes were extracted from 75 g moist soil of +Nem treatments using an automated zonal centrifuge machine (Hendrickx, 1995). After extraction, the nematode suspension was poured onto a sieve and covered with a filter paper for 72 hours so that only viable nematodes migrated towards the water and were collected at the bottom of the plate. All viable nematodes, that passed through the filter were then concentrated and counted using a dissecting microscope (40x). Mass fixation was done by adding hot 4% formaldehyde (65°C) at a ratio of 1:2 (5 ml nematode suspension and 10 ml formaldehyde) and immediately cooling in cold water according to the procedure by van Bezooijen (2006). After six weeks of storage at room temperature, the formaldehyde was tapped off carefully until 1 ml of the nematode suspension remained, into which glycerine
was added in a 1:1 ratio. Slides were made for each sample and at least 100 individual nematodes were identified from each slide to family or when possible to genus level based on Bongers (1994) and assigned to the corresponding functional groups according to Yeates et al. (1993a). However, Filenchus was considered as fungivorous as suggested by different authors (Buchan et al., 2012; Christensen et al., 2007; Okada et al., 2002) and the remaining genera of the Tylenchidae family considered as root feeders.

Maturity and community indices (channel, structural and enrichment indices) of nematodes were calculated according to Bongers (1999) and Ferris et al. (2001). Each nematode taxa identified in the sample was first classified into a colonisers-persisters (cp) scale (from cp1=colonizers to cp5 persisters) based on their natural attributes such as reproduction rate according to Bongers (1999). The maturity index (MI) is the weighted mean frequency of all free-living nematodes excluding plant parasitic nematodes. MI2-5 is a maturity index excluding enrichment opportunist (cp1) nematodes. The plant parasitic index (PPI) is the weighted mean frequency of all plant parasitic nematodes. Nematodes classified as cp2 were considered basal (b) to both enrichment (EI) and structure indices (SI) (Ferris et al., 2001). Bacterial-feeding cp1 and fungivores in cp2 were considered as indicators of enrichment (e). Nematodes of all feeding habits classified in cp3-5 are indicators of structure (s). At the beginning of the experiment, nematodes were extracted from the unirradiated control soil in order to compare with the irradiated samples in terms of abundance and diversity (i.e. a check of the success of reinoculation).

5.2.2 Microbial Biomass C and PLFA

Microbial biomass C and changes in the structure of the microbial community were determined using the same procedure explained in the previous Chapters. Unlike previous Chapters, the changes in the microbial community structure were determined at each sampling date.

5.2.3 Enzyme activities

The procedures to determine the dehydrogenase activity and β-glucosidase activity were the same as explained in previous Chapters.
5.2.4 Mineral nitrogen, phosphorus and sulphur dynamics

N mineralization in the soil samples was monitored throughout the incubation by measuring mineral nitrogen (NH$_4^+$-N and NO$_3^-$-N) as explained in Chapter 3. Water extractable P was measured according to the procedure by Self-Davis et al. (2009). Briefly, 20 ml of demineralized water was added to a centrifuge tube containing 2 g of air dry soil. After 1 hour shaking, the samples were centrifuged at 6000 rpm for 10 minutes. The solution was then filtered through a 0.45 µm membrane filter and acidified to pH 2 with concentrated HCl (38%) to avoid precipitation of phosphate compounds. Finally, inorganic P in the filtrate was determined colorimetrically at 882 nm (Varian Cary 50 spectrophotometer) following a procedure by Murphy and Riley (1962).

Extractable SO$_4^{2-}$-S was determined using 0.01M CaCl$_2$ based on Houba et al. (2000). Briefly, 10 g of air dried soil and 100 ml 0.01M CaCl$_2$ was added to polyethylene tubes and put on a shaker for an hour. Part of the solution was centrifuged at 1800 rpm for 10 minutes. The SO$_4^{2-}$-S in the filtrate was then measured by Ion Chromatography (Metrohm 761 compact IC).

5.2.5 Plant nitrogen, phosphorus and sulphur uptake

All the grass in each core was carefully removed from the soil (including the roots) by gently shaking the roots to detach the soil as explained in Gebremikael et al. (2014b). The above ground biomass was then briefly rinsed in distilled water to remove dust and other contaminants. The roots were further rinsed in distilled water for an extra few minutes to make sure that soil particles were completely detached. The plant samples were then dried in an oven for 48 hours at 60°C and ground down to a mean particle size of 0.01 mm. Total dry biomass was measured before grinding as the sum of the weights of both roots and shoots.

N and S uptake in the grass was considered as the sum of total N and S in the roots and plant biomass determined separately by Elemental analysis (Variomax CNS analyzer, Elementar, Germany). N uptake was calculated as: (%N in the plant sample multiplied by the total dry plant biomass collected at each sampling time) and converted to µg N g$^{-1}$ dry soil. S uptake was calculated the same way as N uptake. P uptake was determined in the aboveground
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biomass only as the root biomass was not sufficient for analysis. Total P in the samples was extracted by a combination of ashing and wet digestion with 1N HNO₃. 1 g of the dry plant biomass was transferred into porcelain cups and put in a muffle furnace at 450°C for 4 hrs. After the calcination, the ash was carefully transferred into a 100 ml glass cup by rinsing with about 20 ml of 1N HNO₃ and two times with distilled water. The solution was then boiled until the volume of the liquid was less than 20 ml. After cooling, the liquid was filtered through Whatman 589/3 filter paper. The total P concentration was determined using the colorimetric Nitro-vanado-molybdate method of Hogue et al. (1970) using a Varian Cary 50 spectrophotometer at 425 nm.

5.2.6 Statistical analysis

Two way analysis of variance (ANOVA) was applied as the experiment followed a factorial design with two fixed factors: Factor 1) nematodes with two levels: +Nem and –Nem, Factor 2) incubation time with six levels: 0, 7, 24, 47, 67 and 105 days of incubation. The normality and homoscedasticity of the data was checked for all the parameters and log transformation was done for the variables that violated the assumptions. Whenever the interaction between nematodes and time was non-significant (p>0.05), the main effects were compared and Fisher’s least significant difference (LSD) was used to analyze mean differences. However, when the interaction term was significant (p<0.05), a one-way ANOVA model was fitted for each nematode-time combination and Bonferoni’s method was used for the post hoc pairwise mean difference analysis. Two tailed bivariate correlation was carried out on selected variables. The PLFA data were further analyzed by Principal Component Analysis (PCA) based on the correlation matrix after checking the sampling adequacy using the Kaiser-Mayer-Olkin (KMO) test. All statistical analyses were done using IBM SPSS Statistics 20 software (SPSS inc., Chicago, USA)

5.3 Results

5.3.1 Nematode abundance and community structure

The abundance and community composition of nematodes in reinoculated samples was similar (18.0 vs 18.3 ind. g⁻¹ dry soil) to the non-irradiated fresh control samples. All the nematode taxa identified in the fresh unirradiated soil that was used as a control sample (CTR)
were also identified in the reinoculated samples. (see Table 3.1) The total abundance and the abundances of each feeding group (except carnivores) significantly increased (p<0.05) over time in reinoculated samples. Only the bacterivores significantly increased from day 7 to day 47 and from day 47 to day 105, while fungivores and herbivores significantly increased only from day 47 to day 105. At the beginning of the incubation, both herbivores (43%) and bacterivores (47%) were dominating the trophic composition, however, at the end the incubation herbivores (55%) significantly dominated over bacterivores (28%) and fungivores (15%), even though the latter two groups significantly increased in abundance over time (Figure 5.1).

**Figure 5.1.** The evolution of mean nematode abundances in each trophic group over time in reinoculated samples (+Nem). The -Nem treatment is not presented as this treatment was without nematodes. The error bars indicate standard error of the mean (n=3).

The plant parasitic index (PPI) did not significantly change over time (Table 5.2). Maturity index (MI) significantly increased over time, but MI2-5 did not significantly change. The channel (CI) and basal (BI) indices significantly increased over time, while the enrichment (EI) and structural (SI) indices significantly decrease over time.
Table 5.1 Evolution of nematode indices over time for the +Nem treatments. Indices were calculated according to Bongers and Ferris (1999) and Ferris et al. (2001) as explained in section 5.2.4. The data corresponds mean values (n=3) and standard error of the mean.

<table>
<thead>
<tr>
<th>Days</th>
<th>PPI</th>
<th>MI</th>
<th>MI 2-5</th>
<th>EI (%)</th>
<th>SI (%)</th>
<th>BI (%)</th>
<th>CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>7</td>
<td>2.57 ± 0.01a</td>
<td>1.55 ± 0.04a</td>
<td>2.14 ± 0.08a</td>
<td>82.39 ±1.46a</td>
<td>20.99±11.51a</td>
<td>16.77±1.00a</td>
</tr>
<tr>
<td>+Nem</td>
<td>7</td>
<td>2.52 ± 0.03a</td>
<td>1.55±0.07a</td>
<td>2.11 ± 0.06a</td>
<td>82.01±1.91a</td>
<td>18.35±9.67a</td>
<td>17.11±2.17a</td>
</tr>
<tr>
<td>+Nem</td>
<td>47</td>
<td>2.51 ± 0.06a</td>
<td>1.73±0.05a</td>
<td>2.10 ± 0.03a</td>
<td>69.21±2.27a</td>
<td>16.61±4.20a</td>
<td>29.15±5.23a</td>
</tr>
<tr>
<td>+Nem</td>
<td>105</td>
<td>2.55 ± 0.03a</td>
<td>1.94±0.02b</td>
<td>2.01 ± 0.01a</td>
<td>39.13±9.19b</td>
<td>2.01±1.00b</td>
<td>60.12±3.82b</td>
</tr>
</tbody>
</table>

PPI= plant parasitic index, MI= maturity index, MI2-5= maturity index without early colonizers (cp1) nematodes, EI= enrichment index, SI= structural index, BI= basal index, CI= channel index

5.3.2 Microbial biomass and community structure

The microbial biomass C was generally lower in +Nem than in –Nem treatments throughout the incubation (Figure 5.2a). Unlike Cmic, a significant statistical interaction was observed between incubation time and nematode treatment on total PLFA (p<0.05)(Table 5.2). +Nem treatments resulted in lower total PLFA than –Nem treatments at the initial periods of incubation, but total PLFA was higher in +Nem than –Nem during the rest of the incubation period (Figure 5.2b). Significant differences only occurred at day 24 and 47. Unlike total PLFA, no significant interactions were observed between time and nematode treatment for marker PLFAs of G+, G- and actinomycetes and no significant differences in average abundances over time between +Nem and –Nem treatments (Table 5.3). Like for the total PLFA, significant interactions between time and treatments were observed for the marker PLFAs of saprophytic fungi, arbuscular mycorrhizal fungi and bacteria to fungi ratio (B:F). Saprophytic fungi and AMF marker PLFA concentrations in +Nem were significantly higher than in –Nem on day 24 and 47 (fungi) and on day 67 and 105 (AMF). B:F did not differ statistically in +Nem treatments and –Nem during the initial and final sampling days of the incubation period, but was significantly lower in +Nem at 24th and 47th days of incubation.

PC1 contributed 36.0% and PC2 25.4% of the total variation in the data set as analysed by principal component analysis (Figure 5.3). PC1 did not clearly separate the treatments except –Nem from that of +Nem at t2 and t3. PC1 was mainly loaded with non-signature long chain
fatty acids such as C22:0 and signature PLFAs for microbial groups, particularly fungi and actinomycetes (Figure 5.3; Appendix 4). PC2 mainly separated the treatments based on the incubation period. It clearly separated the initial period of the incubation (t0 and t1) from the rest of the incubation period. Unlike PC1, PC2 was mainly loaded by signature PLFAs such as G+ bacteria and saprophytic fungi.

5.3.3 Enzyme activities

Dehydrogenase activity was lower in +Nem than in –Nem samples throughout the incubation period, except at the end of the experiment (Figure 5.2c). The average dehydrogenase activity over the incubation period was significantly lower in +Nem samples than –Nem samples (mean difference = 10.2 µg TPF g⁻¹ soil day⁻¹, p<0.05).

Betaglucosidase activity was also lower in +Nem than in –Nem samples throughout the incubation period, except in the initial and the final stage of the experiment (Figure 5.2d). The average betaglucosidase activity over the incubation period was significantly lower in +Nem samples than –Nem samples (mean difference = 11.4 µg PNP g⁻¹ soil day⁻¹, p<0.01).
The role of nematodes in N, P and S availability in planted microcosms with an organic amendment

Figure 5.2 The evolution of mean (n=4) Cmic (a), total PLFA (b), dehydrogenase (c) and betaglucosidase activities (d) over time in +Nem and –Nem treatments. The error bars indicate standard error of the mean (n=3).
The role of nematodes in N, P and S availability in planted microcosms with an organic amendment

<table>
<thead>
<tr>
<th>PLFA</th>
<th>Biomarker for</th>
<th>Loading on PC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C22:0</td>
<td>Not specific</td>
<td>0.90</td>
</tr>
<tr>
<td>C23:0</td>
<td>Not specific</td>
<td>0.90</td>
</tr>
<tr>
<td>C24:0</td>
<td>Not specific</td>
<td>0.89</td>
</tr>
<tr>
<td>C160</td>
<td>Not specific</td>
<td>0.87</td>
</tr>
<tr>
<td>C18:2ω9</td>
<td>fungi</td>
<td>0.85</td>
</tr>
<tr>
<td>10MeC17</td>
<td>Actinomycetes</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PLFA</th>
<th>Biomarker for</th>
<th>Loading on PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>aC15:0</td>
<td>G+</td>
<td>0.93</td>
</tr>
<tr>
<td>C18:1ω9c</td>
<td>Fungi</td>
<td>0.91</td>
</tr>
<tr>
<td>C14:0</td>
<td>Not specific</td>
<td>0.91</td>
</tr>
<tr>
<td>iC15:0</td>
<td>G+</td>
<td>0.89</td>
</tr>
<tr>
<td>iC14:0</td>
<td>G+</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Figure 5.3. Mean PLFA scores (n=4) in –Nem and +Nem treatments as analysed by the principal component analysis. PLFAs that contributed most to the variations explained in the PCs are presented in the table. The sampling date is indicated with the letter “t” plus a numerical value (0, 1, 2, 3, 4 or 5) corresponding to 0, 7, 24, 47, 65 and 105 days after the start of the incubation.

5.3.1 Total plant dry biomass and nutrient uptake

Total dry biomass (root and shoot dry weight) was higher in +Nem than –Nem samples throughout the incubation (Figure 5.4d) and significantly so on day 47 and day 105 with mean differences of 129 and 216 kg ha⁻¹, respectively. Significant statistical interaction was found between incubation time and nematode treatment (p<0.05).
Table 5.2 P values of each parameter based on two way ANOVA for the two factors (treatment and time).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Treatment</th>
<th>Time*Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$-N</td>
<td>0.000</td>
<td>0.365</td>
<td>0.644</td>
</tr>
<tr>
<td>NO$_3^-$-N</td>
<td>0.000</td>
<td>0.066</td>
<td>0.131</td>
</tr>
<tr>
<td>Total mineral N</td>
<td>0.000</td>
<td>0.23</td>
<td>0.78</td>
</tr>
<tr>
<td>Plant and soil N</td>
<td>0.000</td>
<td>0.045</td>
<td>0.026</td>
</tr>
<tr>
<td>Water extractable P</td>
<td>0.000</td>
<td>0.864</td>
<td>0.058</td>
</tr>
<tr>
<td>Plant and soil P</td>
<td>0.000</td>
<td>0.001</td>
<td>0.009</td>
</tr>
<tr>
<td>SO$_4^{2-}$-S</td>
<td>0.000</td>
<td>0.054</td>
<td>0.021</td>
</tr>
<tr>
<td>Plant and soil S</td>
<td>0.000</td>
<td>0.037</td>
<td>0.037</td>
</tr>
<tr>
<td>Plant N</td>
<td>0.000</td>
<td>0.001</td>
<td>0.094</td>
</tr>
<tr>
<td>Shoot P</td>
<td>0.000</td>
<td>0.004</td>
<td>0.00</td>
</tr>
<tr>
<td>Plant S</td>
<td>0.000</td>
<td>0.106</td>
<td>0.89</td>
</tr>
<tr>
<td>Dry biomass (Kg ha$^{-1}$)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.012</td>
</tr>
<tr>
<td>Total PLFA</td>
<td>0.051</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>C$_{mic}$</td>
<td>0.000</td>
<td>0.178</td>
<td>0.35</td>
</tr>
<tr>
<td>Dehydrogenase activity</td>
<td>0.000</td>
<td>0.019</td>
<td>0.632</td>
</tr>
<tr>
<td>Betaglucosidase activity</td>
<td>0.000</td>
<td>0.001</td>
<td>0.101</td>
</tr>
</tbody>
</table>

Nitrogen uptake was significantly higher in +Nem treatments than in –Nem treatments throughout the incubation period (Figure 5.4a). Phosphorus uptake also tended to be higher in +Nem treatments than in –Nem treatments, but only significant so on day 47 and day 105, with mean differences of 3.92 and 3.60 µg P g$^{-1}$ soil, respectively (Figure 5.4b). Significant statistical interaction was found between incubation time and nematode treatment (p<0.05). No significant differences were found in S uptake between +Nem and –Nem treatments (p>0.05) (Figure 5.4c).
Table 5.3 The evolution of microbial groups as determined by marker PLFAs in nmole g⁻¹ dry soil. The values are mean (n=3) ± the standard error of the mean. The symbol * denotes significant differences between +Nem and –Nem on the corresponding sampling dates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (Days)</th>
<th>Total PLFA</th>
<th>G+ve</th>
<th>G-ve</th>
<th>Fungi</th>
<th>AMF</th>
<th>Actinomycetes</th>
<th>Eubacteria</th>
<th>B:F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Nem</td>
<td>0</td>
<td>52.12±215</td>
<td>11.13±0.26</td>
<td>14.50±0.63</td>
<td>5.97±0.34</td>
<td>1.91±0.07</td>
<td>2.51±0.07</td>
<td>38.11±1.40</td>
<td>6.40±0.18</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>52.06±2.23</td>
<td>10.13±0.54</td>
<td>15.95±0.72</td>
<td>5.67±0.18</td>
<td>1.82±0.14*</td>
<td>2.53±0.10</td>
<td>39.92±1.81</td>
<td>6.92±0.11</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>39.22±1.26</td>
<td>7.34±0.39</td>
<td>12.19±0.31</td>
<td>3.63±0.15*</td>
<td>1.48±0.02</td>
<td>2.54±0.05</td>
<td>30.15±1.18</td>
<td>8.21±0.22</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>37.31±8.09</td>
<td>7.50±1.16</td>
<td>12.36±3.20</td>
<td>3.43±0.77*</td>
<td>1.49±0.07</td>
<td>2.83±0.44</td>
<td>28.95±6.61</td>
<td>8.37±0.38</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>57.24±6.60</td>
<td>8.61±0.92</td>
<td>14.14±1.53</td>
<td>10.02±1.32</td>
<td>1.26±0.11*</td>
<td>2.78±0.32</td>
<td>39.48±4.38</td>
<td>3.89±0.11</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>48.20±2.14</td>
<td>8.88±0.37</td>
<td>14.36±0.54</td>
<td>4.96±0.34</td>
<td>1.51±0.02*</td>
<td>2.69±0.13</td>
<td>36.83±1.42</td>
<td>7.34±0.26</td>
</tr>
<tr>
<td>+ Nem</td>
<td>0</td>
<td>48.07±3.46</td>
<td>10.56±0.54</td>
<td>12.18±0.73</td>
<td>5.37±0.44</td>
<td>1.78±0.11</td>
<td>2.32±0.07</td>
<td>34.37±2.15</td>
<td>6.48±0.18</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>46.33±1.45</td>
<td>8.49±0.19</td>
<td>15.35±0.57</td>
<td>4.71±0.17</td>
<td>1.50±0.02*</td>
<td>2.19±0.01</td>
<td>35.65±1.25</td>
<td>7.52±0.03</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>58.87±1.83</td>
<td>8.93±0.24</td>
<td>14.25±0.53</td>
<td>10.54±0.43*</td>
<td>1.51±0.05</td>
<td>3.04±0.06</td>
<td>39.57±1.25</td>
<td>3.70±0.04</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>57.35±3.02</td>
<td>8.46±0.67</td>
<td>14.45±0.76</td>
<td>9.55±0.50*</td>
<td>1.61±0.13</td>
<td>2.72±0.23</td>
<td>38.50±2.01</td>
<td>4.04±0.08</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>60.55±3.09</td>
<td>8.90±0.36</td>
<td>15.19±0.74</td>
<td>10.26±0.51</td>
<td>1.66±0.08*</td>
<td>2.82±0.14</td>
<td>41.40±2.07</td>
<td>4.01±0.05</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>52.11±1.70</td>
<td>8.35±0.23</td>
<td>16.04±0.54</td>
<td>5.82±0.09</td>
<td>2.21±0.07*</td>
<td>2.61±0.10</td>
<td>38.51±2.00</td>
<td>6.61±0.13</td>
</tr>
</tbody>
</table>

P (nematodes)  
0.81  0.36  0.96  0.83  0.58  0.96  0.63  0.86

P (time)  
0.05  0.00  0.17  0.00  0.00  0.08  0.220  0.00

P (nematode*time)  
0.00  0.11  0.27  0.00  0.00  0.34  0.03  0.00
5.3.2 N, P and S dynamics

The sum of mineral nutrients extracted from the soil and nutrients taken up by the plant is considered as the total available nutrient concentration (i.e. the sum of what was initially present in the soil and what was mineralized during the experiment).

NH$_4^+$-N and NO$_3^-$-N concentrations in the soil were not significantly different between +Nem and −Nem treatments. However, the total N availability (the sum of mineral N in the soil and N taken up by the plants), was significantly higher in +Nem treatments than in −Nem treatments on day 42 and day 105 with mean differences 15.3 and 9.62. µg N g$^{-1}$ soil, respectively (Figure 5.5a). The net N mineralized which was calculated as the difference in total available N at the start (t0) and end of the incubation was 45.4 and 56.8 mg N kg$^{-1}$ soil in −Nem and +Nem, respectively, i.e. about 25% higher in +Nem.

Like the mineral N, water soluble inorganic P (Pi) concentration in the soil was not significantly different between +Nem and −Nem treatments (Figure 5.5b). The total available P (the sum of inorganic P in the soil and P taken up by the plants), was significantly higher in +Nem treatments than in −Nem treatments on day 47 and final sampling time, with mean differences of 4.07 and 3.18 µg P g$^{-1}$ soil, respectively. The net available P which was calculated as the difference in total available P at the start (t0) and end of the incubation was 21.4 and 26.5 mg N kg$^{-1}$ soil in −Nem and +Nem, respectively, i.e. about 23% higher in +Nem.

Unlike N and P, sulphate concentration in the soil was significantly higher in +Nem than in −Nem treatments during the initial weeks of the incubation period. The total S availability (SO$_4^{2-}$-S) in the soil + plant S) was also significantly higher in +Nem than −Nem on day 7 and 24, with mean differences of 10.3 and 6.4.µg SO$_4^{2-}$-S g$^{-1}$ soil, respectively (Figure 5.5c)
Figure 5.4. The evolution of mean plant uptake of nitrogen (a), phosphorus (b), sulphur (c) and mean grass dry biomass (d) over time in +Nem and −Nem treatments. The data refers to the cumulative nutrient taken up by the entire plant biomass (root+leaves) until each sampling time. The error bars indicate standard error of the mean (n=4).
Figure 5.5. The evolution of total nutrient mineralization expressed as the sum of what was available in the soil and what was taken up by the plants at each sampling date for N (a), P(b) and S(c) in +Nem and –Nem treatments. The error bars indicate standard error of the mean (n=4).
5.4 Discussion

The primary objective of this experiment was to quantify the contribution of the entire free-living nematode community to N, P and S mineralization and uptake under conditions of high nutrient availability. High nutrient availability was created by applying a grass-clover amendment at a rate which provided 0.13 mg N and 1.4 mg C g\(^{-1}\) dry soil. Our previous work showed that nematode abundance was significantly higher in clover amended soil in comparison to soil amended with farm compost and slurry and unamended soil (Buchan, 2013; Gebremikael et al., 2014b). Nematode indices were computed to study the response of functional groups of nematodes to the grass-clover amendment and change in community structure during the course of decomposition. The differences in nutrient availability and plant uptake between +Nem and –Nem treatments is discussed in relation to the structural changes in microbial and nematode communities. Like the previous Chapters, the contribution of nematodes was considered as the simple differences between +Nem and –Nem treatments as the sole difference between these two treatments is the absence or the presence of nematodes.

5.4.1 Nematode abundances and community structure

Nematode reinoculation was successful both in terms of abundance and diversity. The main purpose of the non-irradiated control soil (CTR) was to compare the reinoculated samples with the field condition in terms of nematode abundance and community structure at the start of the incubation. A further comparison during the incubation period was not done as the CTR soil entails nematodes and all the other fauna while +Nem comprises only nematodes. The nematode abundance was almost similar in +Nem (18.0 ind. g\(^{-1}\) soil) and CTR (18.3 ind. g\(^{-1}\) soil) on day 7 of the incubation. All the taxa that were identified in the CTR were also identified in the +Nem samples, showing the representativeness of the simulated microcosms to the field condition in terms of nematode abundance and community structure. Moreover, all the nematode indices of the +Nem samples were comparable to the CTR except for the CI which was about 50% higher in +Nem as compared to CTR at the beginning of the incubation (Table 5.1). As the objective of the experiment was to study the collective
The role of nematodes in N, P and S availability in planted microcosms with amendment

collection of nematodes, the high degree of resemblance of the reinoculated samples to the field condition (CTR) is a crucial achievement.

The total nematode abundance and the abundance of each group (except carnivores) significantly increased over time in +Nem samples (Figure 5.1). In the absence of plants, nematode abundance often decreases or shows no change over time (Buchan et al., 2013; Gebremikael et al., 2014b; Xiao et al., 2010). This is logical as the food source for microbes and nematodes may get depleted over time in bare microcosms. Even with the addition of an organic amendment such as clover in bare microcosms, nematode abundances decreased by 38% after 97 days of incubation, suggesting that even the effect of added residues on nematode abundance is only short lived (Buchan, 2013). However, in the presence of plants, nematode abundance increased 144% in unamended cores (Gebremikael et al., 2014b) and 417% with amendments in the current study. Such significant increase in abundance in planted microcosms could possibly be due to the continuous supply of energy by the plants through exudates and dead roots for both the microbial and nematode community (Bardgett et al., 1999a; Bardgett et al., 1999b; Denton et al., 1999; Yeates et al., 1999a). The additional energy supplied through grass-clover amendment could be responsible for the further increase of nematode abundance in the current amended soil. The plants in amended microcosms in the current study also showed better growth (about four fold biomass) than the previous study without amendment. The rapid grass growth may also have stimulated the growth of herbivores directly.

The maturity index without cp1 (MI2-5) did not change over time, but the MI including cp1 significantly increased over time suggesting that enrichment opportunist cp1 nematodes such as rhabditids were responsible for this change in the MI (Table 5.1). In line with our hypothesis and previous findings by Ferris and Bongers (2006), both enrichment (EI) and structure indices (SI) decreased over time showing that the community became less enriched and less structured. The nematode community structure was almost equally dominated by herbivores (43%) and bacterivores (47%) at the start of the incubation. However, at the end the incubation, herbivores nematodes dominated (55%) the nematode community followed by
bacterivores (28%) and fungivores (15%). This is in line with our previous findings that the presence of plants change the nematode community structure in favour of herbivores (Gebremikael et al., 2014b). However, the changes seem to occur gradually and only towards the end, as on day 47, the relative and absolute abundance of herbivores and bacterivores was similar (43 vs 44%). The explanation for this could be that enrichment opportunists such as Rhabditidae may have increased at a similar pace as herbivores until the added OM was depleted and decreased afterwards. The decrease in enrichment index (EI), which is a measure of opportunist bacterivores and fungivores nematodes further supports this hypothesis.

The nematode channel index (CI) which is an indication of predominant decomposition pathways significantly increased over time indicating that the fungal channel was dominating over time. This is partly supported by the PLFA data which showed a significant increase of fungal and AMF abundances. In agreement to this, fungivorous nematodes also rapidly increased towards the end of the experiment (about fivefold increase in abundance between day 47 and day 105) while bacterivores increased two fold. Data of feeding groups should be carefully interpreted as the feeding classification of some of the nematodes into feeding group is not consistent. For example, despite the experimental evidence that *Filenchus* are fungivorous (Okada and Kadota, 2003), different authors considered them as root-feeder (Neher, 1999) or as a ‘facultative root-feeder’ (Okada and Harada, 2007) suggesting that they could switch their feeding strategy. Here, *Filenchus* were considered as fungivores.

### 5.4.2 Microbial biomass, community structure and enzyme activities

The total PLFA concentration which is considered as a proxy for and indicator of an active microbial biomass (Bardgett et al., 1999a) was higher in +Nem than −Nem during most of the incubation period. The interaction between herbivorous nematodes and plants may increase the microbial biomass and activity as infestation by plant feeding nematodes may increase the release of root exudates which boosts the microbial growth as shown by several authors (Bardgett et al., 1999a; Bardgett et al., 1999b; Denton et al., 1999; Yeates et al., 1999a). Microbivorous nematodes may also increase the active biomass through several mechanisms.
such as microbial turnover and phoretic transport (Freckman, 1988). Unlike previous reports by Bailey et al. (2002) and Gebremikael et al. (2014b) there was no significant correlation between $C_{\text{mic}}$ and PLFA ($r=0.24$, $p>0.05$) in the present experiment. The presence of nematodes did not significantly affect the microbial biomass $C$.

There was no significant difference between +Nem and –Nem treatments on the abundances of the major bacterial groups (G- and G+) and actinomycetes, showing that the presence of nematodes in planted microcosms did not change the bacterial community structure. This is in agreement with our findings in similar experimental set ups but without amendment. Other studies reported shifts in bacterial community structure based on a few selected species of bacterivores which are not comparable with the current study (Djigal et al., 2010; Irshad et al., 2011; Mao et al., 2007; Xiao et al., 2010). In the current study, the abundances of fungi and AMF and B:F ratio varied over time as there was significant interaction between nematode treatments and incubation period. The saprophytic fungal abundance was significantly higher in +Nem on day 24, indicating that the presence of the entire free-living nematode community positively influenced fungal community at least temporarily. Previous studies reported a higher fungal abundance in clover amended microcosms without plants in samples reinoculated with the entire free-living nematode community (Buchan, 2013). AMF was higher in +Nem treatments than –Nem towards the end of the incubation period and was also significantly correlated ($p<0.05$) with herbivores ($r=0.81$), bacterivores ($r=0.9$) and fungivores ($r=0.79$) suggesting that the interaction between plants and nematodes positively influenced the AMF. Nematodes may increase root development and branching (Mao et al., 2007) and the increased availability of N due to nematodes may enhance the N nutrition of AMF that may eventually increase the biomass of AMF (Koller et al., 2013b). B:F ratios varied over time showing that both bacterial and fungal decomposition pathways were alternatively dominating during the decomposition process regardless of the presence of nematodes. The bacterial channel generally dominates immediately after the addition of external resources to decompose the labile part, and is followed by fungal pathways decomposing the more recalcitrant parts. However, Strickland and Rousk (2010) in their recent review paper revealed that this is not always the case due to several factors such as functional redundancy, C:N ratio
of the litter and nutrient availability. The current study based on a long term (105 days) incubation and B:F determinations at several stages of the decomposition, provides additional evidence that the decomposition pathways may not be always predicted based on C:N ratio of the substrate.

PCA also did not generally segregate +Nem and −Nem treatments, except at t2 and t3. At these sampling dates (t2 and t3), there were also significant differences in total PLFA, fungal PLFA and B:F ratio between +Nem and −Nem treatments. Moreover, PC1 was mainly loaded by non-signature PLFAs and actinomycetes and fungi, suggesting that the presence of nematodes influenced these microbial groups temporarily. PC2 separated the initial period of the incubation from the rest of the incubation period. The loadings suggest that some bacterial and fungal biomarkers were dominating the microbial community (Figure 5.3; Appendix 4). This was further confirmed by the evolution of the microbial group over time, for example, G+, fungi and AMF biomarkers significantly changed over time (Table 5.3). There is no previous report to compare with, whether the inoculation of the entire nematode community changes the microbial community or not, except our own previous research. Indeed, in a similar experimental set up, but without amendment, we found comparable results suggesting that the presence of the entire free-living nematode community in planted microcosms did not shift the microbial community structure (except a temporal shift in fungal community).

Both the dehydrogenase and betaglucosidase activities were generally lower in +Nem than in −Nem throughout the incubation period. To the best of our knowledge this is the first report on the interaction effect of nematodes and plants on enzymatic activities. Dehydrogenase is an indicator of the general microbial activity (Moeskops et al., 2010). The presence of nematodes was expected to increase dehydrogenase activity as nematodes are reported to increase particularly the abundances and activities of bacteria, directly through grazing and indirectly through supplying energy from the roots (Bardgett et al., 1999a; Ingham et al., 1985). However, the dehydrogenase activity did not increase and was not significantly correlated to neither total PLFA nor Cmic.
Betaglucosidase, an enzyme that is involved in C mineralization (Alef and Nannipieri, 1995), was also expected to be higher in the presence of plants as it is produced by nematodes, although fungi are the most important source (Esen, 1993). In contrast to previous suggestions, the increase in fungal abundance and the presence of nematodes did not increase betaglucosidase activity.

5.4.3 Plant nutrient uptake and dry biomass yield

The interaction between nematodes and plants increased N and P uptake throughout the incubation. Only a few studies, with contrasting results, are available on the influences of nematodes on N and P uptake to compare with. Ingham et al. (1985) found increased N uptake in grass shoots in the presence of a bacterial feeding nematode species. In their experiment the fungal feeding nematode species showed no effect on N uptake, but on P uptake. Djigal et al. (2004) also found a significant increase in N and P uptake in maize plants in the presence of a single species of bacterivorous nematode as compared to the control. In contrast to this, Bardgett and Chan (1999) found no significant effect of bacterivorous nematodes on N and P uptake of Montane grassland. The entire nematode community also did not show a significant increase in N uptake of rye grass in unamended soil in a similar experimental setup as the current study (Gebremikael et al., 2014b).

Here, in line with our hypothesis, nematodes increased nutrient availability, plant nutrient uptake and subsequent plant growth under resource rich conditions. This could be due to the combined effects of increased nematode abundance over time and shifts in community structure of both microbes and nematodes in +Nem treatments. Several authors have reported that microbivorous nematodes excrete nutrients that are in excess of their metabolic need in the form of NH$_4^+$, PO$_4^{3-}$ and low molecular weight compounds such as amino acids (Ferris et al., 2012; Ingham et al., 1985). The abundances of the main microbivorous nematodes (both bacterivorous and fungivorous) significantly increased in number following the addition of grass-clover amendment (Figure 5.1). As explained above, previous studies reported that cp1 and cp2 nematodes such as Rhabditidae and Aphelenchidae that feed on bacteria and fungi, respectively, dominate the nematode
community following enrichment by e.g. the addition of organic matter. Such a rapid growth of bacterivorous nematodes may possibly have resulted in a higher nutrient release and the subsequent plant nutrient uptake and growth. The presence of high and significant correlation coefficients between bacterivores and both N uptake ($r=0.67$, $p<0.05$) and P uptake ($r=0.93$, $p<0.01$) further supports this hypothesis. Plants compete for the nutrients that become available by the grazing effect of these nematodes which otherwise would be reimmobilized by the microbes (Bardgett and Chan, 1999).

In addition to the microbivorous nematodes, herbivores also significantly increased in number over time. Unlike the previous experiment, the herbivorous community here comprised sedentary endoparasitic nematodes such as *Meloidogyne* and *Pratylenchus* which were proven to cause the release of more root exudates and produce enzymes that facilitate organic matter decomposition through partially degrading cellulose (Tu et al., 2003) as compared to ectoparasitic nematodes. Studies demonstrated that *Meloidogyne* infection increased N, P and K concentrations in aboveground plant parts and sometimes in the roots (Karssen and Moens, 2006.). The other possible explanation for this enhanced N and P uptake could be the higher and increased abundance of AMF which were shown to increase both P and N uptake (Johansen et al., 1996). In the current study, AMF abundance were significantly higher in +Nem than in –Nem on day 65 and 105.

Although, the presence of nematodes did not significantly enhance plant S uptake, the total S availability (SO$_4^{2-}$ in the soil + S taken up by the plant) was significantly higher in +Nem than –Nem, on day 7 and on day 24. There are no literature data on the contribution of nematodes to S cycling in terrestrial ecosystems. There are, however, several studies on aquatic ecosystems that revealed the interactions of nematodes with sulphur oxidizing bacteria influencing the S cycle (review by Freckman (1988)). The positive temporal influence of nematodes on S availability in the current study could be explained through excretory compounds from the nematodes such as proteins, peptides and free amino acids that are released into the soil and which are also easily mineralizable sources of S (Ferris et al., 2012; Wright and Newall, 1980).
The dry biomass yield was significantly higher in the presence of nematodes, in line with our expectation. Previous studies reported that the presence of selected bacterivorous nematodes increased plant biomass. Djigal et al. (2004) found a 12% increase in maize plant biomass in the presence of bacterial feeding nematodes as compared to the control. Ingham et al. (1985) also found that the presence of nematodes increased plant growth, particularly in the initial stages of growth. The 9% increase in dry biomass was comparable with these previous findings, although the experimental set up was not comparable. Nematodes did not show a significant increase in dry biomass yield in our previous similar set up without amendments, suggesting that the role of nematodes in increasing plant biomass depends on the C and N availabilities in the soil. Presumably, under conditions of low nutrient availability, microbes may win the competition for nutrients against roots. In such conditions plants may not benefit from the presence of nematodes as was reported in Chapter 4.

5.4.4 N, P and S dynamics

Both mineral N and inorganic P concentrations in the soil showed no significant difference in +Nem and –Nem treatments. In planted microcosms, mineral N and P concentrations in the soil alone do not represent the mineralization process because nutrients that become available are continuously taken up by plants. In such cases, nutrient mineralization should be measured as the increase in total nutrient availability i.e. the sum of mineral nutrients available in the soil and the nutrients taken up by plants. In the current study total available N and P were significantly higher in +Nem than -Nem treatments, showing the real effects of nematodes on nutrient mobilization. The increase in net N (25%) and net P (23%) availability in +Nem as compared to –Nem in this study is in the same order of magnitude as with the generally estimated contribution of soil fauna to N mineralization (Verhoef and Brussaard, 1990). The increased N availability may have also been enhanced by the addition of amendments with low C:N ratio, as amendments with low C:N (e.g. 10.7 in this experiment) are generally expected to reduce immobilization of N. Unlike N and P, the sulphate concentration in the soil was significantly higher in +Nem than in –Nem, and also the total S mineralized followed the same trend. Higher S availability was observed only during the initial periods suggesting a temporal influence of nematodes (Figure 5.5c).
5.5 Conclusions

The interactions between the entire free-living nematode community, native microbes and plants in grass-clover amended microcosms significantly enhanced nutrient uptake, particularly N and P. We have also shown here for the first time that nematodes significantly influence S mineralization temporarily, although the plant S uptake was not significantly influenced by nematodes. Unlike previous reports, which suggested that the contribution of nematodes to nutrient mineralization is only of significance at low nutrient availability, here we found an increase of about 25% and 23% in net N and P mineralization under conditions of high nutrient availability. The presence of nematodes did not increase enzymatic activities nor changed the microbial abundance and community structure as such, suggesting that the increased mineralization and plant N and P uptake could be through direct release of nutrients by microbial grazers instead of indirect contribution through stimulating microbes.
Chapter 6:

General discussion and conclusions


6.1 Introduction

Studies have shown that nematodes contribute to OM decomposition, nutrient cycling and plant nutrient uptake through several mechanisms such as nutrient release, stimulating microbial growth and activity and modification of plant root architecture. Because most of these findings are based on a few selected species of nematodes and research was often carried out in the absence plants, the contribution of the entire nematode community to the above mentioned ecosystem functions is poorly understood. The aim of this thesis was to quantify the collective contribution of the entire indigenous free-living nematode community to nutrient mineralization, plant nutrient uptake and microbial properties at different levels of interaction.

First, we sought to quantify nematode contribution to C and N mineralization in the absence of plants. Based on a literature survey, we hypothesized that collectively, nematodes increase C and N mineralization significantly in both amended and unamended microcosms. We also aimed to quantify their collective contribution to mineralization including additional levels of interactions, i.e., in the presence of plants in amended and unamended microcosms. Here, we hypothesized that the nutrient mineralization process and plant nutrient uptake would be enhanced due to these multitrophic interactions. In this concluding Chapter, the research questions and the related hypotheses presented in Chapter 1 are summarized into the following three topics, and any significant findings will be further synthesized and discussed in relation to the large body of literature on belowground soil biological interactions:

i. Does the experimental setup allow to study the true interactions between the nematode community, native microflora and plants?

ii. How do nematodes affect the microbial properties at different levels of interaction?

iii. How do these interactions influence the collective contribution of nematodes to nutrient cycling and plant nutrient uptake?
6.2 Does the experimental setup reliably represent the true interactions in the rhizosphere?

One of the primary objectives of this thesis was to develop realistic experimental conditions that at least allow interactions between the principal soil organisms that are responsible for OM decomposition and nutrient cycling. These organisms primarily include bacteria, fungi and their grazers (protozoa and nematodes) and plants. Nematodes are probably the best candidate to be included in set-ups simulating the rhizosphere as they possess key positions in the soil food web and profoundly interact with other organisms including plants. Herbivorous nematodes directly interact with plants as they feed on roots. Bacterivorous and fungivorous nematodes feed on the primary decomposers i.e. bacteria and fungi, omnivorous and predatory nematodes feed on multiple sources including protozoa (de Ruiter et al., 1993; Hunt et al., 1987). Nematodes are probably the most important fauna that regulate the bacterial, fungal and root decomposition channels (Ferris et al., 2012; Zhao and Neher, 2014) and have also been found to graze more bacteria than did protozoa in the rhizosphere regions of several plants (Griffiths, 1990a, b). Food web models estimated that bacterial feeding nematodes and protozoa contribute 83% of the total N mineralization (Hunt et al., 1987).

Bacteria and fungi generally comprise more than 90% of the soil microbial biomass and are the key regulators of OM decomposition (Six et al., 2006). Thus, experimental set ups need to ensure that bacteria and fungi are accurately represented in the soil microcosms both in terms of abundance and diversity. Soil ecologists often sterilize the soil completely and inoculate it either with a selected species of bacteria or fungi (Anderson et al., 1983; Griffiths, 1994; Ingham et al., 1985) or with the ‘entire’ bacterial and fungal community extracted from the soil (Xiao et al., 2010) or just add dung in an effort to simulate the microbial community (Bardgett and Chan, 1999). The latter two methods seem to be effective in creating a microflora community that is representative of field conditions. However, no efforts have been made to compare the inoculated microcosms against untreated fresh soil to verify whether inoculation was successful in making samples representative of the field condition.

The microbial communities reinoculated in sterilized soil samples, potentially differ from indigenous microbial community in terms of abundance, community structure and spatial location they occupy. Literature has shown that obtaining a representative and quantitatively
reproducible extractions of bacteria is very challenging. This is primarily due to the fact that 80% of bacteria are preferentially located in the micro pore spaces and that microorganisms are also known to produce extracellular polymeric substances that promote the irreversible adhesion of cells to soil particles (Bockelmann et al., 2003; Ranjard and Richaume, 2001). Studies have also shown that the bacterial population introduced into the soil occupies significantly different locations than the indigenous bacteria (Recorbet et al., 1995). Such different locations may potentially lead to overgrazing by nematodes in reinoculated samples as the spatial distribution of the reinoculated microbes in inoculated samples would make these more accessible to nematodes than in the untreated control soil (Bonkowski and Clarholm, 2012; Xiao et al., 2010).

For these reasons, avoiding inoculation and leaving the original microflora intact in soil microcosms seems a crucial prerequisite for accurately quantifying the contribution of soil fauna and in particular nematodes to nutrient mineralization. However, this has not been possible and has not been taken into consideration in most of the previous studies, partly due to the absence of appropriate methodologies that leave the microflora intact. Djigal et al. (2004) avoided inoculation of the microflora, and reported the use of native microflora after subjecting the soil to a 7 fold cycle of freezing and thawing at -18 °C and room temperature, respectively. However, the microbial abundance and structure in the defaunated samples was not compared to untreated fresh control soil.

In this thesis and in recently published studies (Buchan et al., 2013; Buchan et al., 2012), the application of gamma irradiation as a defaunating tool while keeping the microflora intact has been optimized and verified by comparing reinoculated samples against a fresh control soil. Buchan et al. (2012) recommended 5 kGy gamma irradiation to eradicate nematodes completely while leaving the microbial biomass and structure largely intact. This dose resulted in two major unwanted side effects: a decrease in the microbial biomass (about 20%) and a nutrient flush (NH$_4^+$-N was about nine fold higher in irradiated than in the control soil) (Buchan, 2013; Buchan et al., 2012). The reduction in microbial biomass was not statistically significant, and the microbial community structure as determined by PLFA profiles was not affected at this dose. The nutrient flush in irradiated microcosms may influence the OM decomposition process and the role of soil fauna, as microbial activity is largely inhibited by the availability of C and N (Huhta, 2007; Osler and Sommerkorn, 2007). We made further
efforts to standardize this method, particularly in terms of moisture content at the time of irradiation and reduce the nutrient in the flush following gamma irradiation.

Our findings clearly indicated that moisture content at the time of irradiation had effect on eliminating nematodes and on other microbial parameters, most likely due radiolysis effects as suggested by several authors in previous studies (McNamara et al., 2007). This radiolysis effect could partly explain the discrepancies in recommended doses for complete removal of nematodes and shows the need to take the moisture content at the time of irradiation into consideration, particularly at the lower doses.

Leaching with water seems partly successful in reducing total mineral N as only 9 and 7% higher total mineral N was found in leached samples irradiated at 3 and 5 kGy dose, respectively than in unirradiated control samples. However, the average NH$_4^+$-N concentration in leached samples was still many folds higher than in the control, showing that the ammonium flush remained high in leached samples. We did not expect to reduce NH$_4^+$-N concentration directly by leaching with water, but rather indirectly through reducing the substrate (dissolved organic compounds). The concentration of dissolved organic compounds was not measured in leached samples, but the data on NO$_3^-$-N indicated that the reduced mineral N in leached samples was due to leaching of nitrate. Nutrient flushes in gamma irradiated soil remain an important issue for further investigation.

In this thesis, the application of 3 kGy on samples with 80% WFPS moisture content at the time of irradiation was chosen as an optimum dose in making representative microcosms to study interactions between microbes and nematodes compared to 5 kGy at 80% WFPS moisture content. The main reasons for this were complete eradication of nematodes and leaving comparable microbial biomass and community structure to the CTR as determined by PLFA analysis. No significant differences were found in total PLFA and biomarker PLFAs for major bacterial groups (G+ and G-) and saprophytic fungi. Despite previous findings (Buchan et al., 2012; Gebremikael et al., 2014b), the application of 5 kGy only eradicated nematodes at 80% WFPS moisture content and resulted in significant differences in microbial groups. The application of 5 kGy at lower moisture content (50% WFPS) did not did not completely eradicated nematodes and as a result it was not further used in this thesis. This is partly explained by the exact dose applied in our experiment (which was 4.5 kGy) suggesting the
General discussion and conclusions

need to check the exact dose applied during irradiation, and to check whether the applied dose eliminates the target organism.

Despite our partial failure to reduce the nutrient flush following the application of gamma irradiation, arguably it is to be preferred above other soil sterilizing/defaunating techniques such as autoclaving and freeze drying, particularly in terms of microbial biomass. Perhaps, every sterilization technique impacts microbial biomass. Here, the advantages of gamma irradiation were shown by leaving a representative native microflora and avoiding the need to perform reinoculation. In a series of experiments that we conducted, the use of these lower doses preserved native microbial biomass and community structure close to these present in field conditions. Our findings suggest that leaving native microflora through the application of gamma irradiation may lead to more realistic estimates of the roles of soil nematodes and other fauna in soil processes in general and may be forwarded as a potential method in related themes of research in soil ecology.

6.3 How do nematodes affect soil microbial properties at different levels of interactions?

Several studies investigated the interaction between microbes and nematodes, but as explained in the introduction, previous studies were often limited to a selected species of bacteria and bacterivorous nematodes. The influences of these selected nematodes on microbial biomass has never been consistent. For instance, the presence of bacterivorous nematodes decreased microbial biomass by 18% (Bouwman et al., 1994) and 28% (Djigal et al., 2004) as compared to the control (no nematodes) in bare and planted microcosms, respectively. On the other hand, Bardgett et al. (1998) reported a 62% increase in microbial biomass in the presence of bacterivorous dominated nematode communities over microcosms without nematodes.

A reduced microbial biomass in the presence of nematodes is often attributed to overgrazing (Bouwman et al., 1994; Buchan, 2013; Buchan et al., 2013; Djigal et al., 2004) and increased microbial biomass is often attributed to both the phoretic activity and nutrients excreted by nematodes. Microbes are moved from resource poor microsites to substrate rich microsites either on the cuticle of nematodes or after ingestion and passage through the intestine (with a large proportion of bacteria defecated live) (Anderson et al., 1983). This mechanism was
supposed to work more in bare microcosms than in microcosms with rhizosphere as in the latter resources are more evenly distributed and nematodes may not benefit from the movement as such (Standing et al., 2006). In the rhizosphere, low densities of root infestation by herbivorous nematodes have been consistently shown to increase microbial biomass through the release of exudates (Bardgett et al., 1999b; Denton et al., 1999; Dromph et al., 2006; Haase et al., 2007; Moeskops et al., 2010; Yeates et al., 1998).

In contrast to our hypothesis and these findings, the presence of nematodes did not generally significantly influence the microbial biomass in series of experiments conducted in this thesis, regardless of the presence of plants or amendments. The possible explanations for the absence of this expected positive interaction effect of nematodes on $C_{mic}$ could be over grazing by nematodes and less infestation by root herbivores (thus less root exudate release).

Interactions between nematodes and microbial community in bare and planted microcosms without amendment did not change the microbial community structure as determined by total and marker PLFAs (Chapter 4). In grass-clover amended bare microcosms also the influence of nematodes on microbial community structure was not significant (Chapter 3). Further interactions of nematodes with plants in grass-clover amended microcosms also did not significantly change bacterial community structure but made some significant changes in the fungal community structure (Chapter 5). In contrast to our findings of no significant effects of nematodes on bacterial community, bacterivorous nematodes have been shown to systematically increase the nitrifying (Xiao et al., 2010) and decrease the denitrifying bacterial communities (Djigal et al., 2010) and to change the general microbial community structure (Griffiths et al., 1999) in bare microcosms.

The change in the fungal community in our experiment (Chapter 5) was significant although it was only temporal (fungi at 24 and 47 incubation days and AMF after 64 and 105 days). The B:F ratio was also significantly varying over time showing the switch between bacterial and fungal decomposition pathways. Previous studies reported a shift in microbial community structure, particularly increased fungal abundance and F:B ratio by low level infestation of *Meloidogyne* species of nematodes (Haase et al., 2007). The bacterial channel is generally expected to dominate in amendments with a lower C:N ratio such as grass-clover, however, no clear-cut relationship exists between C:N ratio and B:F ratio. Recent studies show that
fungi may dominate during organic residue decomposition with low C:N (Buchan, 2013; Gusewell and Gessner, 2009; Strickland and Rousk, 2010). Our findings support the growing evidence that fungal community may benefit from organic residues with low C:N in the same fashion bacterial community does. It has been suggested that functional redundancy may exist between bacteria and fungi (Strickland and Rousk, 2010).

Although microbial biomass is positively correlated to C and N mineralization, studies based on microscopic observation suggested that only a small portion of bacteria (between 15-30%) may be active (Clarholm and Rosswall, 1980). In addition to PLFA, which shows active biomass, we determined enzymatic activities in the presence of the entire free-living nematode community. However, both dehydrogenase activity (which is indicative of overall microbial activity) and betaglucosidase activity (an enzyme that is involved in C mineralization) were not significantly different in the presence of nematodes compared to their absence regardless of the amendment and plants.

The generally accepted mechanism explaining how nematodes contribute to OM decomposition and nutrient cycling is stimulation of microbial activity either by increasing the microbial community or by favouring selected species of the microbial community such as nitrifiers. Our findings show that this is not always the case and depends on the level of interactions. Fungal community and the corresponding B:F ratio was influenced by the entire nematode community only in planted microcosms amended with grass-clover, i.e. under conditions of high nutrient and energy availability.

6.4 How do interactions between microbes, nematodes and plants influence nutrient cycling and plant nutrient uptake?

Several studies have long acknowledged the roles of nematodes in nutrient cycling. Most of these findings are based on simplified experiments often using a few selected species of nematodes and microbes excluding many possible interactions. Although these findings contributed much to our knowledge and understanding of the roles of nematodes in important ecosystem functions, little is known on how these interactions influence nutrient cycling. We kept interactions in the microcosms as close as possible to the field conditions by keeping the native microflora and inoculating the entire nematode community, adding residues and plants. The influence of nematodes on nutrient cycling was then investigated at
different levels of biotic and abiotic interactions. The lowest level of interaction being between native microbes, the entire free-living nematodes and indigenous organic matter (Chapter 3 and Chapter 4), was compared against added organic matter, particularly with respect to C mineralization (Chapter 3). The level of interactions became more complex by adding plants in these microcosms without amendment (N mineralization and N uptake, Chapter 4) and further with grass-clover amendments (N, P and S mineralization and plant uptake, Chapter 5).

**C mineralization**

Here, the aim was to quantify the collective contribution of nematodes to C mineralization under contrasting C and N availabilities through direct measurement of CO$_2$-C in microcosm experiments. In contrast to our hypothesis, nematodes did not show significant effect on C mineralization in amended or unamended soils. We also measured other related parameters such as Cmic, PLFA, dehydrogenase and enzymatic activities at the end of the incubation. The presence of nematodes did not show any significant effect on all these parameters as well. Selected species of bacterivorous nematodes were experimentally shown to respire most of the C assimilated as CO$_2$ often after feeding on bacteria (Chen and Ferris, 1999; Marchant and Nicholas, 1974). A few other studies that tested bacterivorous nematodes in soil microcosms amended with simple organic amendments such as glucose, also found about 50% increase in CO$_2$-C by a single species of bacterivorous nematodes (Coleman et al., 1978b). Contrasting findings in this thesis may be explained by the differences in experimental setups with these previous studies. Other studies estimated that nematodes may contribute between 0.8 to 2% of the heterotrophic respiration in different forest ecosystem (which is about 10-15% of the contribution of the total fauna (Sohlenius, 1980). In these cases respiration was not measured directly, but estimated indirectly using oxygen consumption. We are reporting for the first time the collective effects of nematodes in realistic experimental set-ups. Our data indicate that the contribution of nematode to overall C mineralization is negligible. Unlike to C, the contribution of nematodes to N mineralization was significant in amended soil. Nematodes mineralize N because of their high respiration and C:N ratio differences between them and their prey. The first case is not supported in our study, as no significant effect on respiration was found. We suggest because of the differences in C:N ratio, nematodes may have excreted
excess N without significant effect on C. Moreover, in amended soil, there could be sufficient N which reduces immobilization and thus increasing net N mineralization.

**N dynamics and plant N uptake**

Without plants, the collective contribution of nematodes to N mineralization (+32%) was comparable to the estimates made by different soil food web models. The contribution of bacterivorous nematode species to N mineralization and N uptake have been verified experimentally in soil microcosms during previous studies (Ferris et al., 1998; Ingham et al., 1985; Woods et al., 1982). In the bare microcosm study (Chapter 4), the nematode community was dominated by bacterivorous nematodes (ca. 70%), suggesting that most of the additional N mineralized was because of these bacterivorous nematodes. The presence of nematodes consistently influenced nitrification regardless of amendments in bare microcosms (Chapter 3 and 4) in line with previous findings (Buchan et al., 2013; Xiao et al., 2010).

Although the underlying mechanism how nematodes are involved in the nitrification process is not clearly understood yet, two possible mechanisms have been suggested. The first possibility could be that nitrifying bacteria may benefit from phoretic activity of nematodes. The other possibility could be a shift in microbial community structure due to nematode grazing. Previous studies indicated a change in ammonia oxidizing bacteria community composition (*Nitrosomonas sp* dominated) in the presence of bacterial feeding nematodes (Xiao et al., 2010). To verify whether a shift in AOB and/ or phoretic activity was the case in our experiment as well, we analysed the AOB community by denaturing gradient gel electrophoresis (DGGE) based on PCR amplification of the amoA gene. The presence and intensity of bands did not show differences between the presence or absence nematodes (data not shown). Our findings in N dynamics clearly showed remarkable differences in the activity of nitrifies in the presence of nematodes, but the DNA analysis could not detect this difference. Further analysis with molecular techniques such as qPCR and/or genes sequencing may be needed to compare the abundance and diversity of nitrifying community.

In the presence of plants without amendments, an additional contribution of nematodes to N mineralization and uptake was not observed (Chapter 4). This was in contrast to our expectation and to the large body of literature that reported the enhancement of N mineralization and plant N uptake as a result of interactions between microbes, fauna and
plants (Bardgett et al., 1999b; Djigal et al., 2004; Ingham et al., 1985; Irshad et al., 2011). However, further interactions of nematodes, microbes and plants in grass-clover amended microcosms, enhanced net N mineralization and plant N uptake (+25%) in comparison to the absence of nematodes (Chapter 5). The mechanisms that have been suggested to explain how interaction between plants and microbes and their faunal grazers enhances N mineralization and uptake in soil ecosystems are both nutritional (e.g. microbial loop) and non nutritional (e.g. root proliferation and microbial community structure shift).

According to the microbial loop mechanism, an additional C input due to root herbivory, stimulates microbial growth and activity, which in turn stimulates OM decomposition by microbes and the excretion of more N by grazers into the system eventually. The results in Chapter 4 do not seem to support the microbial loop mechanism, as the presence of nematodes did not increase N mineralization and plant N uptake and as there was no increase in microbial biomass. For a significant contribution of the microbial loop, there should be sufficient root exudate release and sufficient microbial grazers in the system. Studies have indicated that roots release N together with C and this C is only sufficient to allow N recycling that is lost from the roots instead of stimulating N mineralization from the SOM (Griffiths and Robinson, 1992). The experimental evidences that root herbivory may have a significant stimulatory effect through root exudates so far came from a series of experiments using endoparasitic nematodes on Trifolium. In the particular experiment in Chapter 4 with plants in unamended microcosms, the majority of root feeding nematodes were ectoparasitic, and these may not cause significant root exudate release. Moreover, the relative abundance of microbial grazers in these microcosms was small (20% bacterivores) compared to herbivores (about 70%), suggesting that the additional N released by bacterivores could be negligible. Thus, the seemingly low root exudates and the low abundance of nematode grazers indicate that the microbial loop mechanism was not the main mechanism for increased N mineralization in this experiment.

In the presence of grass-clover amendment (Chapter 5); however, the abundance and community structure of nematodes in planted microcosms was different from the previous experiment without amendment (Chapter 4). The herbivorous community comprised some endoparasitic nematodes that may have resulted in more root exudate release stimulating microbial growth. Moreover, the presence of significantly higher (about two fold) abundance
of bacterivorous and fungivorous nematodes in amended microcosms, may have resulted in stronger microbial grazing and the subsequent increase in the plant available nutrient pool (microbial loop). Furthermore, a microbial community structure shift, particularly increased fungal and AMF abundance in the presence of nematodes may have contributed to more N mineralization. In addition to increasing inorganic nutrient uptake, studies have shown the direct role of AMF in OM decomposition and organic nutrient uptake (Schimel and Bennett, 2004). Thus, both the microbial loop mechanism and the shift in the microbial community structure may explain the enhanced nitrogen dynamics in our experiment.

In a recent proposal to incorporate soil fauna into models of soil C and N cycling, the contribution of soil fauna has been presented in two ways. Some faunal groups such as bacterivorous nematodes contribute directly to the mineral N pool and others such as fungal feeding nematodes contribute mostly to the dissolved organic matter (DOM) pool (Osler and Sommerkorn, 2007). As this model suggested, the huge increase in mineral N pool in microcosms in our study in the absence of plants (Chapter 4), could be mainly due to the presence of large abundances of bacterivorous nematodes. It is also possible that the nutrient flush due to gamma irradiation may have boosted the microbial biomass and activity which in turn increased microbial grazing and the subsequent N release to the mineral N pool. This may not be the case in planted microcosms since the nutrients are taken up by plants. Herbivorous nematodes show low assimilation efficiency like the fungivorous (de Ruiter et al., 1993), which means that they also contribute directly to the DOM pool through defecation. Thus, the collective contribution of nematodes to nutrient cycling could be directly to the mineral N pool (mostly by bacterivores and omnivores) and to the DOM pool (by fungivores and herbivores). Most of the biogeochemical models do not consider shifts in microbial community structure such as increased AMF abundance, auxin producing bacteria and nitrifying bacteria. Our findings suggest that as many interactions as possible need to be considered to increase our understanding of the role of soil fauna in nutrient cycling.

P mineralization and plant uptake

The influences of nematodes on P mineralization and plant P uptake is poorly investigated, partly because P release is thought to be controlled chemically more than microbially (Cline et al., 1985). However, it has been acknowledged that microbes are actively involved in the
mineralization and availability of P in the soil, particularly phosphate solubilizing bacteria (De Bolle et al., 2013; Gyaneshwar et al., 2002). In previous controlled experiments, bacterivorous nematodes were shown to contribute to P availability and plant P uptake (Djigal et al., 2004; Irshad et al., 2012; Irshad et al., 2011). In our experiment, the presence of nematodes collectively contributed in 23% more net available P in planted microcosms amended with grass-clover. Although the mechanisms explaining this still need to be investigated, findings from the present and previous studies suggest the importance of the microbial loop and association with AMF fungi for such increased P availability.

**Plant growth and biomass yield**

The interactions between nematodes, microbes and plants have been known to exert influence on plant growth and biomass production. Increase in shoot biomass, particularly by the presence of selected species of bacterivorous nematodes has been recorded in different experiments conducted in vitro and in vivo (Djigal et al., 2004; Ingham et al., 1985). In our study, the collective contribution of nematodes to plant dry biomass yield was significant (+9%) in amended microcosms (Chapter 5). Increased nutrient availability due to the presence of nematodes may lead to increased nutrient uptake and subsequent plant growth. Apart from nutritional effects of nematodes, non nutritional effects through root proliferation as a result of production of plant hormones induced grazing may also be responsible for such increase in plant biomass (Bonkowski et al., 2009; Mao et al., 2006).

On the other hand, nematodes are traditionally known to cause plant biomass losses and negatively affect crop production. In our study, it seems that plants were not negatively affected by the presence of nematodes, probably because the abundance of most important plant parasitic nematodes was below the threshold level to make significant yield losses. Low level nematode infestation, for example by clover cyst nematodes have rather been found to enhance microbial activity and nutrient cycling in grassland ecosystems (Bardgett et al., 1999b). Thus at low level of infestation and in the presence of the entire free-living nematode community, plants may benefit from further interactions of herbivores with microbes and microbial grazers through nutritional and non-nutritional effects, eventually balancing the negative effects.
6.5 Conclusions and future perspectives

This thesis has clearly shown that the collective influence of nematodes on and their contributions to microbial properties, nutrient cycling and plant nutrient uptake varies depending on the level of interactions. For instance, in the absence of plants, the collective contribution of nematodes to C and N cycling was found to be significant. With further plant interaction, an additional effect of nematodes on N mineralization and uptake was only significant in the presence of a readily degradable amendment. However, further important interactions with other fauna, particularly protozoa, collembola, mites and earthworms are missing in the model system we used. Given that collembola and mites feed on fungi and nematodes, presumably influence the overall contributions of nematodes to nutrient cycling. The macrofauna particularly earthworms were found to reduce the abundances of nematodes. Future studies should incorporate other fauna to understand how interactions between different groups of soil fauna affect nutrient cycling by modifying the current experimental set-up.

The mechanism how nematodes are involved in nutrient cycling remain an important gap for our understanding of their role. The existing soil food web models are based on poorly established feeding relationships between soil organisms and their physiological attributes. For instance, *Filenchus* has recently been found to feed on fungi in contrast to previous feeding classification as root feeder. Experimentally determined C:N ratio values of nematodes and their prey are rarely available. Relatively more data is available on respiration efficiency of nematodes. However, our findings (from direct measurement of CO$_2$ respired by nematodes) is in contrast to indirectly estimated CO$_2$ production based on O$_2$ consumption by individual nematodes and their fresh weight. Given that C:N ratio and respiration efficiency of nematodes have been used to explain the mechanism how they are involved in nutrient cycling, further studies are needed to accurately determine these and other physiological attributes. Grazing enhanced production of phytohormones has been suggested as a non nutritional effect of nematodes that may increase plant growth. While such increase in hormones needs further verification, more parameters that regulate plant nutrient uptake may also be necessary for our understanding of how nematodes influence plant nutrient uptake. An increase in DOM pool as a possible mechanism for nematode contribution to nutrient cycling needs to be verified further. Further understanding of the mechanisms how
nematodes are involved in C and N cycling is an important step to incorporate them in C and N cycling models more accurately.

A range of sterilizing/defaunating tools have been used in studying the roles of nematodes and other organisms in nutrient cycling, complicating the comparison and synthesis of available data. A literature survey we made indicated that these techniques have not often been compared against fresh soil, particularly in terms of nutrient flush and microbial community structure. Given the advancement of molecular techniques, it seems important to check how the microbial community is affected by a specific sterilizing techniques, and how the inoculated microflora evolves through time compared to a fresh control soil. At the same time further studies should modify these tools aiming to reduce the unintentional effects such as nutrient flushes.
Summary

Important soil functions and services such as primary production, organic matter decomposition and nutrient cycling are regulated by countless biotic and abiotic interactions in the soil. One of such crucial interactions in relation to Organic matter (OM) decomposition and nutrient cycling is between bacteria, fungi, nematodes and plants. Nematodes are the most abundant multicellular soil organisms which developed a diversified feeding habits. In soil they feed on bacteria (bacterivores), fungi (fungivores), plant roots (herbivores), animals (predators) or a combination of multiple food sources (omnivores). Because of such diversified feeding habits, they occupy key positions in soil food web and regulate energy and nutrient flows. Several studies reported the contribution of nematodes to organic matter decomposition and the subsequent nutrient cycling. However, these findings are based on theoretical food web calculations or on simplified experimental set ups in which a few selected nematode species are inoculated in sterilized soil or inert media and often in the absence of plants. Despite the great contribution of these findings to our knowledge and understanding of the roles of nematodes in nutrient cycling, the findings may be not very realistic and difficult to extrapolate to field conditions. In this thesis we aimed to quantify the influences of the entire free-living nematode community on nutrient cycling and plant nutrient uptake in an experimental set up that allowed various levels of interaction between these nematodes, native microbes and plants.

In the first Chapter, the roles of each functional group in ecosystem functioning were briefly highlighted. Research data on the extent and mechanisms how nematodes influence nutrient cycling and plant growth were assessed in detail. These studies indicated that nematodes contribute directly to the available nutrient pool or enhance nutrient mineralization, availability and plant nutrient uptake by stimulating microbial activity. This chapter also presents a literature analysis on experimental procedures applied in quantifying the roles of nematodes in nutrient cycling, particularly on the advantages and unintentional effects of the common sterilizing or defaunating techniques. These tools were evaluated in relation to the possibility for selective removal of nematodes from the soil whilst minimizing changes in biochemical properties and nutrient flushes. Gamma irradiation was selected over the other methods for this study as it allows to selective removal of nematodes. However, the
unintentional effects of this method are still poorly understood and the method needs to be optimized further.

In Chapter two, an incubation experiment was carried out aiming at further optimizing gamma irradiation as a tool to quantify the contribution of nematodes to nutrient cycling. Particular emphasis was put on the effect of soil moisture at the time of irradiation (radiolysis effect) and the possibility of reducing the nutrient flushes caused by irradiation. Here, leaching the irradiated soil column with water after irradiation was conducted in an effort to reduce the nutrient flush. We found that effects of a given dose (lower than 5 kGy) on soil biochemical properties changed as the moisture content of the soil increases at the time of irradiation. Leaching with water significantly reduced the mineral N concentration, but the NH$_4^+$ concentration remained higher compared to unirradiated control (CTR). The application of a 3 kGy dose in soil samples with a moisture content of 80% Water-filled pore space (WFPS) was found to eliminate nematodes and leave the microbial properties comparable to the CTR. In this way, soil samples were defaunated and reinoculated or not with the entire free-living nematode community resulting in two treatments (with nematodes and without nematodes).

Chapter three presents the experimental setup and findings of an incubation experiment conducted to quantify the collective contribution of the entire free-living nematode community to C mineralization in amended and unamended microcosms. The contribution of nematodes to C mineralization was negligible, as no significant effect were found in cumulative CO$_2$-C their presence. These findings contrasted to our null hypothesis and previous findings that experimentally measure using a bacterivorous nematode. Our data shows that that their overall role in C mineralization is negligible.

In Chapter four, the influences of nematodes on N mineralization from indigenous soil organic matter was investigated in bare microcosms and microcosms planted with rye grass. The presence of nematodes significantly increased N mineralization in bare microcosms. In contrast to previous studies, further interactions of nematodes and microbes with plants did not increase N mineralization. The microbial community structure was not significantly influenced by nematodes in both bare and planted microcosms. However, the nematode community structure was shifted to a dominance of bacterivores and herbivores in the absence and presence of plants, respectively. This change was discussed in relation to the possible link between nematode functional groups and N mineralization.
In Chapter five a similar experimental set up (like in Chapter 4) was used to quantify the influences of nematodes on major plant nutrients (N, P and S) in microcosms amended with fresh grass clover and planted with rye grass. Here, the presence of nematodes significantly increased the availability and plant uptake of N and P and plant biomass throughout a three months incubation period. Nematodes also positively influenced S availability and S uptake, although the effect was only temporal and limited to the initial stage of the incubation. Nematodes did not change the microbial community structure except temporal shifts in fungal community. The abundances and structure of the nematode community changed over time towards herbivorous dominance, but the abundances of microbial grazers also significantly increased over time. These results were discussed in comparison to the experiment in Chapter 4 without amendment.

In the last Chapter, the main findings were discussed and synthesized in relation to the specific themes and research questions of the thesis. The validity and representativeness of the experimental set up to quantify the roles of nematodes in nutrient cycling was discussed. The advantages and limitations of using low dose gamma irradiation in this study were also evaluated in terms of leaving native microflora in the samples and reducing nutrient flushes. The extent and mechanisms how nematodes influence microbial properties and nutrient cycling was discussed at the various levels of interaction determined in this thesis. We concluded that the influence of nematodes on nutrient cycling depends on the levels of interaction considered and the resulting potential shifts in nematode community structure. Finally, we highlighted future research needs to understand the roles and mechanisms further how nematodes regulate crucial soil functions such as nutrient cycling and primary production.
Samenvatting
Belangrijke bodemfuncties en ecosysteemdiensten zoals de primaire productie, de afbraak van organisch materiaal en de nutriëntendynamiek worden gereguleerd door talloze biotische en abiotische interacties in de bodem. Eén van die cruciale interacties met betrekking tot de afbraak van organisch materiaal en de nutriëntendynamiek is de interactie tussen bacteriën, schimmels, nematoden en planten. Nematoden zijn de meest voorkomende meercellige organismen die gediversifieerde voedingshabitats ontwikkeld hebben. In de bodem voeden nematoden zich met bacteriën (bacterivoren), schimmels (fungivoren), plantenwortels (herbivoren), dieren (predatoren) of een combinatie van verschillende voedselbronnen (omnivoren).

Omwille van die gediversifieerde voedingshabitats nemen nematoden in het bodemvoedselweb sleutelposities in en bepalen zij de energie- en nutriëntenstromen. Verschillende studies beschreven de bijdrage van nematoden in de afbraak van organisch materiaal en de daaruit volgende nutriëntendynamiek. Deze bevindingen waren echter gebaseerd op theoretische berekeningen aan de hand van het voedselweb of op vereenvoudigde proefopzetten waarin een beperkte aantal soorten nematoden werd geïnoculeerd in een gesteriliseerde bodem of een inert medium, vaak in de afwezigheid van planten. Ondanks de grote bijdrage van deze bevindingen tot het inzicht in de rol van nematoden in de nutriëntendynamiek, zijn deze wellicht niet erg realistisch en moeilijk te extrapoleren naar veldomstandigheden. In deze thesis trachten we de invloed van de volledige gemeenschap van vrijlevende nematoden op de nutriëntendynamiek en de opname van nutriënten door planten te kwantificeren in een proefopzet die verschillende niveaus van interacties toeliet tussen nematoden, de autochtone microbiële gemeenschap en planten.

In het eerste hoofdstuk werd de rol van elke functionele groep in het ecosysteem kort toegelicht. Onderzoeksgegevens over de mate waarin en de mechanismen waarmee nematoden de nutriëntendynamiek en de plantengroei beïnvloeden werden in detail geëvalueerd. Deze studies wezen erop dat nematoden rechtstreeks bijdragen tot de
beschikbare nutriëntenpool en dat ze de vrijstelling van nutriënten, de nutriëntenbeschikbaarheid en de opname door planten verhogen door de microbiële activiteit te stimuleren. Dit hoofdstuk omvat tevens een literatuurstudie aangaande de experimentele procedures die toegepast worden in de kwantificering van de rol van nematoden in de nutriëntendynamiek, met bijzondere aandacht voor de voordelen en onbedoelde effecten van de gangbare sterilisatie of andere technieken die de bodemfauna selectief elimineren. Deze methodes werden geëvalueerd op basis van de mogelijkheid om nematoden selectief te verwijderen zonder grote nutriëntenflushes en met minimale veranderingen in de biochemische eigenschappen van de bodem. Gammabestraling werd verkozen boven de andere methodes aangezien hierbij de selectieve verwijdering van de nematoden mogelijk is. De onbedoelde effecten van deze techniek zijn echter nog steeds slecht gekend en de procedure dient verder geoptimaliseerd te worden.

In hoofdstuk twee werd een incubatieproef uitgevoerd met als doel de verdere optimalisatie van de gammabestraling als techniek om de bijdrage van de nematoden tot de nutriëntendynamiek te kwantificeren. Er werd in het bijzonder aandacht besteed aan het effect van het bodemvochtgehalte op de bestralingstijd en aan de mogelijkheid om nutriëntenflushes die ontstaan bij de bestraling in te perken. We toonden aan dat de effecten van een zekere dosis (kleiner dan 5 kGy) op de biochemische eigenschappen van de bodem veranderden met toenemend bodemvochtgehalte op het moment van bestraling. De bestraalde bodemkolom werd met water gespoeld om de gevolgen van de nutriëntenflush te minimaliseren. De spoeling verminderde de concentratie aan minerale N significant, maar de concentratie aan NH₄⁺ bleef hoger dan in de niet-bestaalde controlebodem. De toepassing van een 3 kGy dosis in bodemstalen met een vochtgehalte van 80% WFPS bleek nematoden te elimineren zonder een verandering in de microbiële parameters teweeg te brengen. Op deze manier werden bodemstalen selectief ontdaan van nematoden en werden ze nadien al dan niet geïnoculeerd met de volledige gemeenschap van vrijlevende nematoden. Dit resulteerde in een behandeling met en een behandeling zonder nematoden.

Hoofdstuk drie geeft de proefopzet en de resultaten weer van een incubatieproef die uitgevoerd werd om voor de volledige gemeenschap van vrijlevende nematoden de collectieve bijdrage tot de C-mineralisatie te bepalen in bodems met en zonder toevoeging
van organisch materiaal. De aanwezigheid van nematoden verhoogde de C-mineralisatie in bodems zonder toegevoegd organisch materiaal. De C-mineralisatie nam licht af in de aanwezigheid van nematoden in bodems met toegevoegd organisch materiaal (bodems met een hoge nutriëntenbeschikbaarheid). Deze resultaten kwamen niet overeen met onze nulhypothese maar toonden aan dat de rol van nematoden in de C-mineralisatie groter is bij een lagere nutriëntenbeschikbaarheid.

In hoofdstuk vier werd de invloed van nematoden op de N-mineralisatie van autochtoon bodemorganisch materiaal onderzocht in geïncubeerde bodemmonsters met en zonder inzaai van raaigras. De aanwezigheid van nematoden verhoogde de N-mineralisatie significant in de onbegroeide bodems. In tegenstelling tot resultaten uit eerdere studies was er geen toename van de N-mineralisatie door andere interacties tussen enerzijds nematoden en de microbiële gemeenschap en anderzijds planten. De structuur van de microbiële gemeenschap werd niet significant beïnvloed door de aanwezigheid van nematoden, zowel in de onbegroeide als in de begroeide bodems. In de structuur van de nematodengemeenschap werd echter wel een verschuiving opgemerkt: bacterivoren domineerden in onbegroeide bodems en herbivoren domineerden in begroeide bodems. Deze verschuiving werd besproken met betrekking tot het mogelijk verband tussen de N-mineralisatie en de functionele groepen binnen de nematodengemeenschap.

In hoofdstuk vijf werd een gelijkvormige proefopzet (cfr. hoofdstuk vier) gebruikt om de invloed van nematoden op de beschikbaarheid van de belangrijkste voedingsstoffen voor planten (N, P en S) te kwantificeren in bodemmonsters met toevoeging van vers organisch materiaal (grasklavermengsel) en inzaai van raaigras. De aanwezigheid van nematoden verhoogde de biomassa van het raaigras en de beschikbaarheid en de opname van N en P gedurende een incubatieperiode van 3 maanden. Nematoden hadden ook een positieve invloed op de beschikbaarheid en opname van S, hoewel dit effect beperkt was tot de initiële fase van de incubatie. Nematoden veranderden de microbiële gemeenschap niet, met uitzondering van een tijdelijke verschuiving in de schimmelgemeenschap. De samenstelling en structuur van de nematodengemeenschap vertoonden na verloop van tijd een dominantie van de herbivoren, maar ook het relatieve belang van de bacterivoren nam toe. Om het effect
van de toevoeging van vers organisch materiaal te beoordelen, werden deze resultaten vergeleken met de resultaten uit hoofdstuk vier.

In het laatste hoofdstuk werden de belangrijkste bevindingen besproken en samengevat op basis van de specifieke thema’s en onderzoeksvragen van de thesis. De betrouwbaarheid en de representativiteit van de proefopzet om de rol van nematoden in de nutriëntendynamiek te kwantificeren, werd besproken. De voordelen en beperkingen van het gebruik van lage dosissen gammastraling in deze studie werden eveneens geëvalueerd met betrekking tot het behoud van de autochtone microflora en het beperken van de nutriëntenflushes. De mate waarin en de mechanismen waarmee nematoden een invloed uitoefenen op de microbiële eigenschappen en de nutriëntendynamiek werden besproken op verschillende interactieniveaus. We besloten dat de invloed van nematoden op de nutriëntendynamiek afhandt van het beschouwde interactieniveau en van de resulterende mogelijke verschuivingen in de structuur van de nematodengemeenschap. Tot slot belichtten we de noden van toekomstig onderzoek om een beter inzicht te verwerven in de rol van nematoden en de mechanismen waarmee zij cruciale bodemfuncties zoals de nutriëntendynamiek en de primaire productie reguleren.
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References


Appendices

Appendix 1: Loadings of each PLFA on PC1 and PC2 at the beginning and end of the incubation as analyzed by PCA. Only values >0.1 are presented in the table.

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**Appendix 2.** Loadings of individual FAMEs in both amended and unamended microcosm

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Appendix 3. Loadings of individual FAMEs in both bare and planted microcosms

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Appendix 4. Loadings of individual FAMEs in planted microcosms with grass clover amendment

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Curriculum Vitae

Personal

First Name          Mesfin Tsegaye
Family Name         Gebremikael
Place and date of birth Bekoji (Ethiopia), 28-03-1975
Nationality         Ethiopian
Email               mgebremi@gmail.com

Academic Background

- MSc in Physical Land Resources (Soil science), 2008-2010. Gent University, Belgium
- BSc in Agriculture (Crop Production), 2003-2007. Ambo University, Ethiopia

Positions and work experiences

- PhD researcher at Gent university Belgium..........................2010-2014
  Quantifying the contribution of nematodes to nutrient mineralization from the soil organic matter- BOF project
- Graduate assistant at Dilla university Ethiopia....................May 2008 – Sep 2008
  Teaching practical lessons microbiology course for undergraduate students
- Research assistant at Ethiopian Agricultural Research institute... 2002-2008
  Field experimental setups, data collection, household surveys and data analysis

International trainings

- Application of stable isotopes in ecological studies, April 2011. Munchen, Germany
- Selecting ecological indicators for ecosystem services, April 2013. Wexford, Ireland

Conference participations and contributions

- Day of young soil scientists, Brussels, Belgium......Feb 2011.......Oral presentation
- Day of young soil scientists, Brussels, Belgium......Feb 2012.......Oral presentation
- 17th Nitrogen workshop, Wexford, Ireland........June 2012.......Poster presentation
- 4th Euro soil science congress, Bari, Italy..............July 2012.......Oral presentation
- Day of young soil scientists, Brussels, Belgium....Feb 2014.......Poster presentation
- 18th Nitrogen workshop, Lisbon, Portugal..............June 2014.......Oral presentation
- The 1st Global Soil Biodiversity conference, Dijon, France....Dec 2014.......Poster

Awards

- The yearly Prize De Boodt-Maselis for outstanding scholar for the promotion of research in Eremology, Gent University Belgium (Sep 2010)
• VLIR-UOS scholarship for MSc study in Physical Land Resources at Gent University, Belgium
• Yearly Prize for an outstanding undergraduate student in Plant sciences, Ambo University, Ethiopia (Sep 2007)

Publications

Peer reviewed international journals


Book Chapter

Proceedings and books of abstracts


