Succession of nematodes during composting processess and their potential as indicators of compost maturity

Hanne Steel

Promoters:

Prof. dr. Wim Bert (UGent)

Prof. dr. Tom Moens (UGent)

Thesis submitted to obtain the degree of doctor in Sciences, Biology

Proefschrift voorgelegd tot het bekomen van de graad van doctor in de Wetenschappen, Biologie

Dit werk werd mogelijk gemaakt door een beurs van het Fonds Wetenschappelijk Onderzoek-Vlaanderen (FWO)

This work was supported by a grant of the Foundation for Scientific Research, Flanders (FWO)

Reading Committee:

Prof. dr. Deborah Neher (University of Vermont, USA)

Dr. Thomaé Kakouli-Duarte (Institute of Technology Carlow, Ireland)

Prof. dr. Magda Vincx (Ghent University, Belgium)

Dr. Eduardo de la Peña (Ghent University, Belgium)

Examination Committee:

Prof. dr. Koen Sabbe (chairman, Ghent University, Belgium)

Prof. dr. Wim Bert (secretary, promotor, Ghent University, Belgium)

Prof. dr. Tom Moens (promotor, Ghent University, Belgium)

Prof. dr. Deborah Neher (University of Vermont, USA)

Dr. Thomaé Kakouli-Duarte (Institute of Technology Carlow, Ireland)

Prof. dr. Magda Vincx (Ghent University, Belgium)

Prof. dr. Wilfrida Decraemer (Royal Belgian Institute of Natural Sciences, Belgium)

Dr. Eduardo de la Peña (Ghent University, Belgium)

Dr. Ir. Bart Vandecasteele (Institute for Agricultural and Fisheries Research, Belgium)

Acknowledgments

Eindelijk is het zover! Ik mag mijn dankwoord schrijven, iets waar ik stiekem al heel lang naar uitkijk en dat alleen maar kan betekenen dat mijn doctoraat bijna klaar is. JOEPIE! De voorbije 5 jaar waren zonder twijfel leuk, leerrijk en ontzettend boeiend. Maar… jawel doctoreren is ook een project van lange adem, met vallen en opstaan, met zin en tegenzin, met geluk en tegenslag, met fantastische hoogtes maar soms ook laagtes….Nu ik er zo over nadenk en om in een vertrouwd thema te blijven: doctoreren verschilt eigenlijk niet zo gek veel van een composteringsproces, dat bij voorkeur trouwens ook veel adem (zuurstof) ter beschikking heeft. Het zijn beide processen waarop verschillende factoren een gunstig (of ongunstig) effect hebben en dat, indien nodig, bijgestuurd moet worden door drastische (of minder drastische) maatregelen om een zo goed mogelijk eindproduct te bekomen. Sommige van deze factoren zijn onmisbaar, andere hebben een klein maar veel betekenend effect. Laat één ding duidelijk zijn, dit proces is dus geen one-man show. Daarom wil ik heel graag een heleboel mensen bedanken, die hebben bijgedragen aan het welslagen van mijn doctorerings-proces.

Eerst en vooral wil ik mijn promotoren **Wim Bert** en **Tom Moens** bedanken.

Wim, met jou als promotor van mijn thesis ben ik in de nematodenwereld gerold. Daarna kreeg ik, dankzij jou, de kans om mijn onderzoek verder te zetten en te doctoreren. Je bent dan ook de vaste waarde in mijn nematoden- en compostverhaal. Ik wil je bedanken voor je eeuwige en aanstekelijke enthousiasme, je relativeringsvermogen wanneer nodig (dit was meermaals het geval), je wetenschappelijke ingesteldheid, maar ook voor de paraatheid waarmee je eigenlijk altijd tijd had voor vragen en uitleg, voor al het naleeswerk en voor de zeer fijne, menselijke en motiverende manier van samenwerken. Als laatste wil ik je nog ontzettend veel succes wensen met het uitbouwen van je relatief nieuwe carrière als prof van het labo Nematologie.

Tom, hoewel je door omstandigheden een beetje later op de kar van mijn doctoraat sprong, was je bijdrage zeker niet minder belangrijk. Door je iets andere expertise had je vaak een verfrissende kijk op mijn onderzoek. Ik wil je dan ook bedanken voor je vaak kritische, maar terechte opmerkingen, voor al het naleeswerk dat mijn teksten naar een hoger niveau bracht en zeker ook voor je betrokkenheid.

Pamela Fonderie wil ik bedanken voor de manier waarop we de voorbije jaren collega's (en eigenlijk ondertussen ook vrienden) konden zijn. Hoewel onze onderwerpen inhoudelijk niet echt bij elkaar aansloten had ik toch het gevoel dat we een goed team waren. Jij was het eerste aanspreekpunt voor mijn preliminaire ideeën en gedachten, maar ook voor mijn frustraties en tegenslagen. Ik wil je dan ook bedanken voor je hulp op alle mogelijke gebieden en je vele

peptalks. Bij jou kon ik altijd terecht en we hebben samen ongelooflijk veel plezier gemaakt. Ik heb heel veel bewondering voor de manier, waarop jij je gezin met het maken van een doctoraat combineert. Daar kan ik nog veel van leren!

Marjolein Couvreur en **Dennis Vlaeminck** wil ik bedanken voor alle, zeer welgekomen, technische ondersteuning. Jullie helpende handen en praktisch inzicht hebben mij enorm geholpen. Super bedankt daarvoor!

David Buchan wil ik bedanken voor het aanleren van het PLFA-extractieprotocol, de bijhorende tips & tricks en voor de hulp bij de interpretatie van de data achteraf. Ook al had je het zelf ontzettend druk met je eigen doctoraat, je was altijd bereid te helpen waar je kom en dat heb ik enorm geapprecieerd.

Bart Vandecasteele en **Koen Willekens** wil ik bedanken voor de talrijke compoststalen die ik mocht nemen van de professionele composteringsprocessen aan het ILVO en voor de uitwisseling van kennis en ideeën over allerlei compost-gerelateerde ontwerpen. Dankzij jullie voelde ik mij ook een beetje thuis op het ILVO. Bedankt voor de prettige samenwerking.

Lien Boshof, Annelies Scholaert en **Wim Vanbossel** wil ik bedanken voor hun enorme inzet tijdens hun thesisjaar met een mooi eindresultaat tot gevolg en **LiesbetColson** voor de hulp bij het mee opzetten en opvolgen van de composteringsproef in de tuin van Wim.

I would like to greatfully thank the **members of the jury** of my PhD: Prof. dr. Deborah Neher, dr. Thomaé Kakouli-Duarte, Prof. dr. Magda Vincx, Prof. dr. Wilfrida Decraemer, dr. Eduardo de la Peña, dr. Ir. Bart Vandecasteele, Prof. dr. Koen Sabbe. Thank you for your commitment to reading my thesis and for the insightful comments.

Wilfrida Decraemer, Nic Smol en **Inge Dehenin** wil ik bedanken voor de vele uitnodigingen op, zowel wetenschappelijke, als iets minder wetenschappelijke activiteiten in het kader van de master-na-master in Nematology. Het was heel leuk om betrokken te worden bij de boeiende smeltkroes van culturen, ideeën en wetenschappelijke uitdagingen, die de cursus met zich meebrengt en zo ook een beetje deel uit te maken van die grote “familie” nematologen van over de hele wereld.

Ook de niet eerder vernoemde collega’s en ex-collega’s verdienen mijn bijzondere dank. **Wouter, Maxime, Stijn, Mieke, Freija** en **Myriam** bedankt voor de gezellige babbels, de koffiepauzes (liefst met taart) en natuurlijk ook voor de uitzonderlijk fijne werksfeer, die ik waarschijnlijk niet meer veel zal tegenkomen. **Barbara** en **Heleen** bedankt voor het aangename

gezelschap tijdens mijn vele treinritten van en naar Gent. Ik heb jullie gemist de laatste maanden, maar ik hoop dat we nog lang onze traditie van uitstapjes en multiculturele etentjes zullen voortzetten.

Mijn vrienden en vriendinnen wil ik vooral bedanken voor hun niet doctoraat-gerelateerde input waardoor ik naast mijn doctoraatswerk ook nog boeiende, leerrijke en leuke dingen te doen had en op tijd eens kon ontspannen om er daarna weer tegenaan te kunnen.

Zonder de voortdurende steun en aanmoedigingen van mijn ouders en familie zou ik nooit geworden zijn wie ik nu ben en zou dit boekje er niet gelegen hebben.Voilà clichés zijn er om bevestigd te worden, want het is natuurlijk gewoon zo. Moeke, bedankt voor alles! Vake, Arne en Ken, de families Steel, Bergmans en Van Parijs: ik wil jullie bedanken voor jullie interesse in mijn doctoraat gedurende de voorbije jaren. Rune en Sita verdienen een pluimpje voor hun magische vermogen om alleen al via gedachten telkens weer een glimlach op mijn gezicht te toveren.

Het laatste woord van dank heb ik bewaard voor Jan, wiens eeuwige enthousiasme voor mij aanstekelijk werkt. Jan, jouw liefde, gezelschap, vertrouwen en realiteitszin hebben van de voorbije jaren een super leuke en boeiende tijd gemaakt (en zo gaan er uiteraard nog veel volgen!). Bedankt om de voorbije maanden zowel de ideale huisman te zijn als te helpen waar je kon en mij altijd te blijven aanmoedigen. Het moet niet altijd even gemakkelijk geweest zijn om met een doctorandus samen te leven!

Table of contents

1. General Introduction

Modified from:

Steel H and Bert W (2011). Biodiversity of compost mesofauna and its potential as an indicator of the composting process status. Dynamic Soil, Dynamic Plant 5 (Special Issue 2): 45-49.

1.1 CONCEPT OF COMPOSTING AND PRACTICAL USE

Composting is an aerobic, heat-producing, and controlled process by which a wide spectrum of organisms converts a mixed organic substrate into water; carbon dioxide (CO_2); inorganic nutrients; and stabilized sanitized organic matter (humus) (Figure 1.1). In contrast to natural rotting or decomposition, the environmental conditions during composting (e.g., moisture, temperature, substrate composition, and oxygenation) remain controllable (Zucconi and de Bertoldi 1987; Sharma et al. 1997; Baldwin and Greenfield 2009) and the process produces a recognizable heat peak (Kutzner 2000) (see also 1.2). Composting is considered important for sustainable agriculture and partly solves the problem of organic waste treatment (Sharma et al. 1997; López et al. 2008). It is an effective tool for the management of municipal and agro-industrial wastes by converting raw organic materials into a stabilized form, destroying human and animal pathogens and recycling valuable plant nutrients (Naidu et al. 2004). Multiple benefits of compost addition to the soil's physical and chemical characteristics have been reported and include increased soil organic matter content (Termorshuizen et al. 2004) and the subsequent improvement of soil aeration, soil porosity, drainage, and water holding capacity (Cogger 2005; Kuo et al. 2004; Termorshuizen et al. 2004). Furthermore, compost can provide an important source of nutrients for plants by providing readily available nitrogen and by promoting mineralization of compost nitrogen in the soil (Cogger 2005; D'Hose et al. 2012; Zvomuya et al. 2008). Composts are also known for their disease-suppressive activity based on both physicochemical and biological mechanisms (Akhtar and Malik 2000; Bailey and Lazarovit, 2003; Kuo et al, 2004; Oka 2010; Reuveni et al. 2002). Another benefit of compost application is the suppression of weed seeds (De Cauwer et al. 2010, 2011). Therefore, truly functional compost should meet criteria for stability and maturity (see 1.3) before application (Zorpas 2009).

The use of compost significantly impacts the soil fauna and flora (Pfotzer and Schüler 1997). Application of compost enhanced the biological activity of the soil (i.e. higher microbial activity and higher numbers of protozoa and bacterial-feeding nematodes), resulting in enhanced turnover of organic matter and release of plant available nutrients (Forge et al. 2003). In translocating and transforming litter and soil organic matter and in changing the supply of nutrients to plant roots, soil fauna fulfill crucial roles in the nutrient supply and the conservation of the soil structure (Brussaard et al. 2004). Hence, compost application can strengthen the role of the living soil. However, current insight into the consequences of compost addition for the complex interactions in the soil is limited. In addition, soil biota can be considered important soil

quality indicators. The nematode community in particular is strongly influenced by soil physical and chemical properties (Boag and Yeates 1998; Goralczyk 1998). Furthermore, there is considerable evidence that organic amendments cause changes to the whole soil nematode community, affecting the presence and abundance of different nematode groups (Bulluck et al. 2002; Nahar et al. 2006; Renčo et al. 2010). The soil nematode community, in turn, plays an important role in nutrient cycling because of its abundance, rapid life cycle and intricate interactions with soil microbes and predators (Ingham et al. 1985).

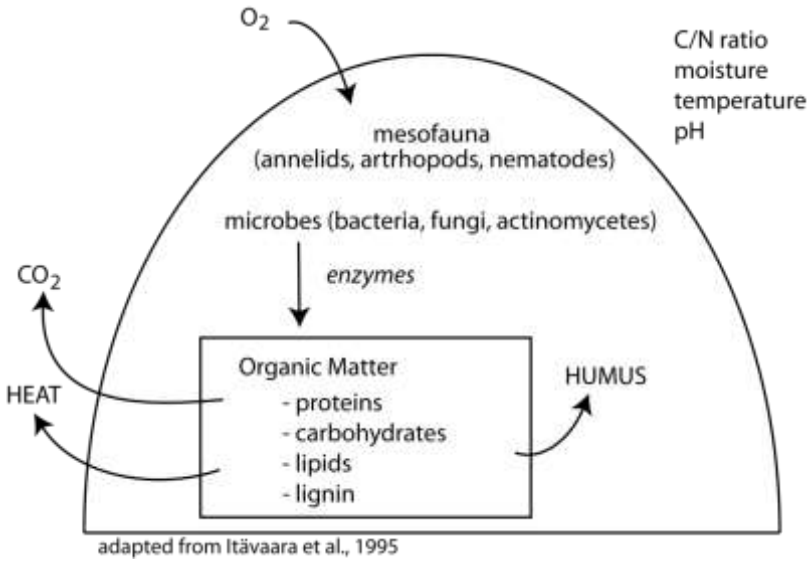


Figure 1.1
Schematic overview of the composting process.

1.2 PARAMETERS AFFECTING COMPOSTING PROCESSES

Under optimal conditions the composting process can be divided into temperature-related phases (Tuomela et al. 2000; Ishii et al. 2000; Ryckeboer et al. 2003b): within a few hours or a couple of days after mixing of the feedstock materials, the thermophilic phase (45-70°C) starts, lasting a few days, several weeks or even months (particularly in food wastes). Afterwards there is a mesophilic or cooling phase with temperatures below 45°C, during which mesophile organisms (often different from those of the feedstock materials) recolonize the substrate. The maturation (or curing) phase is a stabilization phase which can last for several weeks to several months. Temperature is very often not uniform throughout the composting mass with the center tending to be hotter than the peripheral edge. Heat production during composting is almost completely due to heat liberation from microbial metabolisms and is mainly determined by the degradability and energy content of the substrates, the availability of moisture and

oxygen, and the mode of energy conservation (Kutzner 2000; Ryckeboer et al. 2003). An adequate supply of oxygen is needed for aerobic microbial activity and to avoid offensive odours primarily produced by the anaerobic microbial community. Since decomposition predominantly occurs in the thin liquid films on the surface of the organic material, moisture plays an essential role (Kutzner, 2000). If moisture levels drop below a critical level (< 30%) microbial activity will decrease, while a moisture content that is too high (>65%) can cause oxygen depletion and losses of nutrients through leaching (Ryckeboer et al. 2003b). Nitrogen and carbon are the primary nutrients required by the microbial community in composting (Xu 2012). Optimal C/N ratio for active composting has been reported to range between 25 and 35 (Tuomela et al. 2000). If N is limiting during the process, decomposition of plant carbohydrates such as hemicellulose and cellulose will be reduced (Eiland et al. 2001). During composting the C/N ratio decreases due to the release of CO_2 as organic substrates are decomposed and due to the incorporation of N-rich microbial/fungal biomass into the organic substrata. Ammonium (NH_4^+) is released during the rapid degradation of readily available substrates in the beginning of the process and causes the compost pH to increase, generally to above 8 (Wichuk and McCarthney 2010).

1.3. COMPOST MATURITY VS. STABILITY

In order to be considered beneficial for the soil (see also 1.1) compost must be of high quality. Maturity and stability measures are used to evaluate or analyze the composting process and are important parameters for compost quality assessment. Maturity is a general term describing the fitness of the compost for a particular end use, while stability can be defined as the extent to which readily biodegradable material has decomposed (Sullivan and Miller 2001; Gómez et al. 2006). In other words mature compost is ready to use and will not cause adverse effects when used as, or applied to, plant-growing media while stable compost only refers to the resistance of the compost organic matter to further degradation. One of the key issues in compost research is to assess compost maturity as the status of the compost ultimately determines the quality of the product and the associated advantages as mentioned above (Tognetti et al. 2007; Moral et al. 2009). Nevertheless, compost maturity is often loosely defined as the state when compost is dominated by humic substances (Dinel et al. 1996) or as the state where the temperature reaches a near-ambient level (Cooperband 2000). For the past decade, researchers have proposed multiple chemical and physical variables (Zmora-Nahum et al. 2005; Sellami et al. 2008) to assess maturity as well as biological parameters (Gómez et al. 2006). At present and to the best of our knowledge, none of the proposed tests reliably, consistently, and unequivocally

quantifies compost maturity and stability as a stand-alone measure (Rynk 2003; Wichuk and McCartney 2010). Other important characteristics that need to be considered for evaluation of proposed maturity tests are required expertise, necessary lab equipment, time, and costs.

1.4. COMPOST BIOLOGY AND ITS POTENTIAL AS AN INDICATOR OF THE COMPOSTING PROCESS STATUS

1.4.1. State of the art

Despite the fact that the compost fauna is not well known, interest in the ecological effects of composting has been growing and recently soil fauna is proposed as a potential tool to evaluate the ecotoxicology of compost (Kapanen and Itavaara 2001). However, the compost is comprised of fauna that can be used in assessing the maturity and quality of the compost. In general, biological parameters have enormous advantages for monitoring a given system, i.e., they are well correlated with ecosystem functioning, they respond sensitively to management practices and climate, and they illustrate the chain of cause and effect (Doran and Zeiss 2000). The surplus value of biological parameters in system monitoring holds true, especially for compost being the direct result of a biological process.

Compost supports a diversity of microbes (e.g., fungi, bacteria, actinomycetes, and algae), micro-fauna[1] (protozoa), and mesofauna[1] (mainly annelids, arthropods, and nematodes) (Cooperband 2000; Young et al. 2005). The microbes and microfauna are the chemical decomposers responsible for the organic matter decomposition through aerobic respiration, while the larger organisms (mesofauna) are the physical decomposers important for the (initial) mechanical breakdown of organic materials into smaller particles, thereby increasing surface area for microbial action (Cooperband 2000; Young et al. 2005) and/or mesofauna, and occupying key positions in the compost food chain.

Although microbes execute the major share of the primary decomposition, mesofauna have multiple advantages over soil microbes and micro-fauna as indicators for the quality and status of the compost. In general, mesofauna are more integrated in the food web by being one or two steps higher and their response to changes in the environment are more significant on a stable temporal scale because of their longer generation time, this way making it possible to

[1] According to a generalized classification of soil fauna based on body length, microfauna ranges from 0.02 to 0.16 mm while mesofauna ranges from 0.16 to 10.4 mm (from Wallwork, 1970 in Coleman et al. 2004). As such, mesofauna is not synonymous to meiofauna, a term used to describe aquatic benthos retained on sieves with a mesh size of 44 µm but passing through a mesh size of 0.5 mm (Giere 2009), nevertheless both comprise largely the same major taxa, including nematodes.

distinguish between "real" environmental changes and transient nutrient flushes. In addition, some mesofauna groups (e.g., nematodes and mites) occur at more than one level in the energy pyramid and are representative of several levels of consumers.

In this chapter, we provide an overview of the mesofauna found in compost and discuss the potential for each taxon to be an indicator of compost quality and process status. In the second step, the use of effective indicators is further elaborated, based on nematodes as the most promising group.

1.4.2. Mesofauna in compost

Together with bacteria, fungi, and other microbes (e.g., actinomycetes), the mesofauna in compost form a complex food web or energy pyramid with primary, secondary, and tertiary levels of consumers. Obviously, the base of the pyramid or the energy source is made up of organic matter. All the levels above are populated, though not exclusively, by mesofauna. The mesofauna of compost includes Isopoda, Myriapoda, Acari, Collembola, Oligochaeta[2], Tardigrada, Hexapoda and Nematoda. This wide spectrum of organisms forms a complex and rapidly changing community that is not limited to a specific compost but can be found from vermicompost to mushroom compost and from small- to large- scale controlled or open-air composting processes. However, the available information on mesofauna from compost is limited and mainly only present in "grey" literature or from non-scientific works. Information on their presence relative to the stage of a compost process (thermophilic phase, cooling phase, and maturation phase) is virtually absent. This limited information on compost mesofauna, ranging from structuring or natural compost inhabitants to more accidental taxa, will be listed in the following text. The known compost taxa can hereby be roughly classified according to the following main focuses: 1) their direct and visible influence on the compost process (i.e., mainly Oligochaeta) and 2) merely accidental records from general biodiversity studies. According to Coleman et al. (2004) earthworms are strictly macrofauna but for the sake of simplicity they are included in this overview of mesofauna.

Within the phylum Annelida, earthworms occur in diverse habitats with a lot of organic material like manure, litter, and compost (Sharma et al. 2005). Earthworms are very important physical decomposers in the composting process because they maintain the aerobic condition in the compost by tunneling, and therefore mechanically partitioning the organic matter while feeding on this material. As a consequence, they facilitate the transformation of nutrients into available

[2] According to Coleman et al. (2004) some Oligochaeta such as Lumbricidae should be considered as macrofauna but for the sake of simplicity they are here included within the mesofauna.

forms and increase microbial activity (Devliegher and Verstraete 1995; Yakushev et al. 2009). When earthworms are artificially added to a composting process, the process is called vermicomposting. Many recent studies focus on this inoculation with epigeic earthworms and their potential to recycle organic waste materials into value-added products is well documented (e.g., Suthar and Singh 2008). Inoculation with earthworms accelerated the decomposition process (Manna et al. 2003), though the composting potential of different species in diverse compost situations remains to be investigated (Suthar and Singh 2008). In a study on forest litter decomposition by Manna et al. (2003), the composting potential of *Eisenia fetida* was clearly superior compared to other species (i.e., *Perionyx excavatus* and *Dicogaster bolaui*). At present, *E. fetida* is the most popular compost earthworm and is known under various common names, including redworms, brandling worms, and tiger worms. Owing to its recognized function in a compost process, *E. fetida* specimens are widely commercially available in temperate regions for use in vermiculture. Domínguez et al. (2001) investigated the biology and ecology of the earthworm *Eudrilus eugeniae*, which is indigenous in Africa but bred extensively internationally for the fish bait market. These researchers concluded that this earthworm species might be a good candidate for vermicomposting in tropical climates. Thus, earthworms have a well-known positive effect on compost, but they usually only reach high densities after inoculation and/or within specific controlled circumstances. Furthermore, they are not present in all stages of the composting process.

Mites (Acari) are commonly found in compost (Ødegaard and Tømmerås 2000). Smith et al. (1998) and Skoracka et al. (2002) provided an overview of the species found in the investigated composts (respectively in Canada and in Central Europe). Clift and Terras (1995) associated bio-indicator value with mites based on a reported range of mite fauna in most of their investigated mushroom composts. This study reported the presence of either red pepper mites (*Siteroptes mesembrinae*) and/or bacterial feeding mites (*Histiostoma feroniarum*) and associated these mites with the reduced mushroom yield. Clift and Terras (1995) believe these species can serve as an indicator species for poor pasteurization or conditioning in mushroom compost processes. Other encounters with mites (i.e., *Pygmephorus* sp., *Histiostoma* sp., and *Parasitus bituberosus*) in mushroom compost were reported by Al-Amidi (1995). Although the contributions of mites in the compost food web are not known, mites cover multiple levels of the food web, ranging from scavenging on organic debris to fungal-feeding, bacterial-feeding, and predators, and most likely can have a certain structuring effect on the compost process, but this is not yet studied.

Within the arthropods, beetles (Coleoptera) are the most diverse animal group and they can

be found in almost all habitats, including compost (Ødegaard and Tømmerås 2000). Beetles might act as decomposers, fungivores, or as predators on other insects present in the compost (Ødegaard and Tømmerås 2000). Compost heaps can be very rich in beetle species: 273 species were found in compost from Lulea in northeastern Sweden (Lundberg and Persson 1973), >260 species in different composts from Uppsala in central Sweden (Palm 1979), and 225 species in seed-composts from Skane in southern Sweden (Baranowski 1978). A number of exotic beetle species (34) have been established in compost heaps in the Nordic countries during the 20th century. Ødegaard and Tømmerås (2000) hypothesized that the successful establishment of exotic species in compost habitats results from the increased frequency of introductions and warm, thermal stability in large compost heaps during the Nordic winter.

Woodlice (Isopoda) are saprophages that generally live in moist soil, but some species such as *Porcellionides pruinosus* and *P. sexfasciatus* live aggregated in compost and manure heaps (Achouri et al. 2008). Terrestrial isopods are principally consumers of organic debris in an ecosystem. However, according to Messelink and Bloemhard (2007), extreme populations of woodlice may cause serious damage to vegetables. But whereas populations of woodlice increased strongly in fresh compost, in mature compost they barely survived. Therefore, Messelink and Bloemhard (2007) stressed the importance of a complete compost process in order to manage woodlice populations at an acceptable level.

Centipedes (Chilopoda) and millipedes (Myriapoda) are another group of more accidental compost mesofauna. Centipedes are mainly carnivores (Wallwork 1976; Lewis 1981), while millipedes (Diplopoda) feed on plant materials and fragments of decaying organic material, though both groups are found in similar habitats. In urban areas, their common microhabitat is often extended by building stacks of materials like compost and garbage piles (Riedel et al. 2009). The distribution of *Oxidus gracilis* (millipedes) is connected with greenhouses and gardens with compost heaps in higher altitudes (Bergersen et al. 2006). Identified centipedes collected from compost heaps include *Geophilus proximus* and *Haplophilus subterraneus* (Rosenberg and Seifert 1977; Bergersen et al. 2006).

Gutiérrez and Mazo (2004) reported springtails (Collembola) from compost processed at the Corporación Universitaria Lasallista, Colombia. More specific recordings were done by Fjelberg (1998) and Gisin (1960), who found *Hypogastrura manubrialis* and *H. purpurascens* in compost. Nowadays, springtail reproductions in compost products are often used to develop ecotoxicological tests (e.g., *Folsomia candida* (Crouau et al. 2002)) or to investigate the survival of potential biocontrol species after the addition in composts (e.g., *Protaphorura*

armata (Sabatini et al. 2006)).

There also have been more isolated recordings of other Arthropoda taxa in compost. Gutiérez and Vera (2004) found five different families of ants in the compost of the Corporación Univeristaria. Lasallista and Buddle (2010) reported compost heaps as typical habitats for pseudoscorpions. Goulson et al. (1999) found very large calyptrate fly populations associated with composting sites in the United Kingdom, probably caused by the abundance of warm decaying organic matter. Flies are, however, not involved in the decomposition process itself but use the compost heap as a breeding site.

Tardigrades or water bears, exhibit a high tolerance to extreme environmental conditions such as temperature, making them a likely group to appear in compost. However, published recordings of Tardigrada in compost are scarce. Vargha (1995) described *Hexapodibius reginae* from compost and Hatamoto et al. (2008) recorded tardigrades in rice straw compost using DGGE analysis. Our own observations indicate the accidental presence of tardigrades in compost (observations in the framework of this study).

Finally, recordings of nematodes in isolated compost samples were made by several authors, including Andrassy (1983), Anderson (1983), Gagarin (2000), Nadler et al. (2003), and Manso (2004). These primarily bacterivorous nematodes were recorded in general biodiversity studies, but their function within the compost process was not discussed. All the above mesofauna groups can be found in compost, but except nematodes none of them has been consistently found in all investigated compost types. Furthermore none of these groups is hitherto reported to be found throughout all successive compost stages. Considering the population dynamics in relation to the composting process, none of the above taxa have been thoroughly investigated.

1.4.3. Potential of mesofauna as ecological indicator in compost

Several authors have used microbial communities (bacteria and fungi) to evaluate compost maturity. For example, Eiland et al. (2001) analyzed phospholipid fatty acid (PLFA) profiles of compost microbial communities but concluded that there was no correlation with compost maturity. Conversely, Steger et al. (2003, 2005, and 2007) and Ryckeboer et al. (2003a) found typical changes in the microbial community but stressed the need for more studies on different composts. An elaborate overview of bacteria and fungi occurring during composting and self-heating processes is given in Ryckeboer et al. (2003b). However, mesofauna has multiple advantages over microbial communities as indicators for the quality and status of compost. Firstly, it is very difficult and time-consuming to identify all bacteria, fungi, and protozoa in a sample and databases of biochemical profiles are either incomplete or inadequate, especially

for free-living taxa. Secondly, by being one or two steps higher in the food chain, mesofauna serve as integrators of physical, chemical, and biological properties related to their food resources. Third, their generation time (days to years) is longer than that of metabolically active microbes (hours to days), making them more stable temporally and not simply fluctuating with ephemeral nutrient flushes (Neher 2001).

A successful indicator must be able to reflect a past ecological process or predict a future ecological process (Neher and Darby 2009). Therefore, an indicator should have as many as possible of the following characters. For soil condition, the indicator should 1) reflect the structure and/or function of ecological processes in the soil no matter what geographical location is sampled, 2) respond to changes in soil condition, 3) have available methodologies, 4) be comprehensible, and 5) be inexpensive to measure (Neher et al. 2005; Doran and Zeiss 2000). Obviously, a thorough knowledge of the fauna used as an indicator is necessary; however, from the overview, it is clear that the knowledge of compost fauna is only fragmentary. Nevertheless, some mesofaunal taxa present in compost, such as Collembola, mites, earthworms and nematodes, have already proven their potential as bio-indicators (Table 1.1).

Collembola and mites have been widely used to assess the impact of agricultural management practices and disturbances. For example, Barbercheck et al. (2009) reported differences in richness, evenness, and abundances of mites and proportions of collembolans among agricultural sites. Other examples for mites as a bio-indicator include Koehler (1999), Gulvik (2007), and Bedano and Ruf (2010) and for Collembola include Fiera (2009), Chagnon et al. (2000) and Baretta et al. (2008). Despite their potential as bio-indicators, the evaluation of their community assemblages is often limited (O'Neill et al. 2010), due to the high proportion of undescribed species (Coleman and Whitman 2005) and insufficient information on general biology, distribution, and functional roles (Bolger 2001; Greenslade 2007). Geissen and Kampichler (2004) also questioned the suitability of collembolan communities as indicators to determine the influence of amelioration measures on soil ecosystems in European forests because they could not find a relationship after five years of study. This limited literature review shows that there is no univocal consensus about the use of mites and Collembola as bio-indicators in terrestrial ecosystems. Hence, an extrapolation to compost is not evident.

Conversely, the potential of earthworms as indicators of environmental changes is more generally accepted. Their possibilities as bio-indicators are based on community changes (Tondoh et al. 2007) or on a quantifiable bioaccumulation of metals in their tissues (Suthar et al. 2008). However, earthworms do not occur in every composting stage and process and

the accumulation of contaminations is not the main or only focus in assessing the compost status.

Unlike earthworms, Collembola, or mites, nematodes are ubiquitous, even in disturbed or polluted areas (Neher et al. 2005). Nematodes live beside the most diverse and abundant soil organisms and are the most important secondary consumers within soil mesofauna (Mulder et al. 2005). They have been extensively used as indicators of soil biodiversity and functioning (Neher 2001; Mulder et al. 2005) and as indicators of environmental disturbances in soil (Bongers and Ferris 1999; Ferris et al. 2001; Yeates 2003; Cerevková and Renč o 2009). They occupy key positions in the food web because they interact with the species providing their food (e.g., bacteria, fungi, etc.) and other organisms using the same food resources. They are also involved in predator-prey interactions (e.g., protozoa, fungi, and mites as well as nematodes) (Yeates et al. 2009). In these ways, changes in the food web are mirrored in shifts among nematode feeding groups (Yeates et al. 2009). Most importantly, nematodes show a rapid response, translated into proportions of functional (feeding) groups, to changes in the microbial decomposition pathway and the availability of food sources (Yeates et al. 1999; Brimecombe et al. 2001; Zelenev et al. 2004; Sánchez-Moreno et al. 2008), due to their relatively short generation time (Bongers 1990). In addition, the transparent nature of nematodes allows easy observation of the clear relationship between structure and function: their feeding behavior (see 1.4.4) is easily deduced from the structure of the mouth cavity and pharynx (Bongers and Ferris 1999). Furthermore, the relative abundance and small size of nematodes makes sampling relatively easy and cheap. Neher et al. (2005) and Sánchez-Moreno et al. (2009) validated the hypotheses that nematode-based soil food web indices are useful indicators of other soil organisms such as mites. Hence, nematode faunal assemblages theoretically have all of the properties needed to enable an optimal *in situ* assessment of the compost process, based on evaluation and interpretation of their abundance and function.

Table 1.1°
Overview of the mesofauna groups with potential use as bio-indicator and their characteristics.

	Mites	Collembola	Earthworms	Nematodes
OCCURENCE				
- Cosmopolitan/ ubiquitous				X
- Large population size			X*	X
- Present on more than 1 level in food web	X	X		X
FUNCTION & SERVICES IN ECOSYSTEM				
- Important role in N-mineralization				X
- Potential to regulate even suppress magnitude of resources	X		X	X
- Variable sensitivity to stress factors, from extremely sensitive to extremely tolerant			X	X
PRACTICAL APPLICABILITY				
- Easily extracted and cultured				X
- Easy observation				X
- Identification up to species level not needed	X			X
- Inexpensive techniques and methodologies	X	X	X	X

*When artificially added to the process.
°For references see text 1.4.3.

1.4.4 Nematode feeding types

Assignment of nematodes to feeding groups provides insight into the functional role of nematodes in the ecosystem and into the effect of environmental changes on the nematode fauna (Yeates et al. 1993). Although it is clear that nematode feeding types are linked to stoma morphology, inferred feeding strategies without any empirical evidence can be misleading (Yeates et al. 1993). For example similar stoma structures may be used for different feeding behaviours. This is the case within the aphelenchid nematodes were *Aphelenchoides* may be classified as hyphal feeders and ecto- or endoparasites of plants, while the related genus *Seinura* is a predator (Moens et al. 2004). In other cases, species are allocated to one feeding type while they have developmental stages or generations that fit another feeding type (Neher 2001). In addition, the relevance of the (usually non-feeding) free-living stages of animal parasites and of the omnivores (which can be easily allocated to feeding types 3 to 6) for trophic studies has been questioned (Moens et al. 2004). The trophic classification of nematodes in Yeates et al. (1993), which is broadly used and has also been adopted in this study, heavily relies on stoma morphology but has been supplemented to a certain extend with experimental evidence. Yeates et al. (1993) distinguished 8 main feeding types; (1) plant-feeding, (2) hyphal- or fungal-feeding, (3) bacterial-feeding, (4) substrate ingesters, (5) carnivores, (6) unicellular eukaryote feeders, (7) dispersal stages of animal parasites, and (8) omnivores. Not all these feeding types are expected to occur in compost. For example in the absence of living plant tissue, plant feeding nematodes are expected to be absent, which substantially simplifies feeding type allocation of nematodes with stylet or spear.

1.5. AIMS

The overall aim of this study is to contribute to a better understanding of the compost biology during the composting process. Given its potential as bio-indicator for compost, as described above, we especially address the nematode community.

The main objectives of this study are to describe the nematode fauna of a virtually unexplored habitat, to provide the first insights in the nematode succession in a rapidly changing compost environment, to examine the effect of the compost comprising nematodes on the nematodes that are already present in the soil and finally to evaluate the potential of the nematode community as an indicator of compost maturity and quality. These main objectives translate into the following specific objectives:

(i) To acquire fundamental knowledge on the nematode diversity in compost based on morphological analyses of the nematode community, complemented with sequence data. Compare the diversity of compost nematodes with the terrestrial nematofauna in Belgium. Assess whether the same nematode taxa can be found in disparate composting processes using different feedstock materials (Chapter 3).

(ii) To obtain an integrated insight into the succession and the trophic structure of the nematode community during the composting process and link this to a selection of relevant biotic (microbial community) and abiotic factors (time of composting, temperature, pH, moisture content and C/N ratio) (Chapters 2, 4 & 6). This is complemented with experiments to reveal the feeding behavior of one of the dominant nematodes found in most of our composting trials (Chapter 3).

(iii) To assess the survival and colonization capacity of nematodes in compost, by analyzing temperature tolerance and evaluating the importance of insect phoresy[3] for nematode arrival at the composting process (Chapter 5).

(iv) To test the bio-control potential of compost with its associated nematodes by assessing the (short term) development of a soil nematode community after application of mature compost – comprising nematodes – to this soil. Does the species composition of the compost affect the nematode community in the soil? For instance, will the number of plant-parasitic nematodes in the soil decrease and the number of predators and fungal-feeding nematodes increase? (Chapter 7).

[3] Insect phoresy or vectoring is a form of commensalism; a relationship where nematodes use mostly Arthropoda for dispersal or shelter but have no direct trophic relationship with them (Timper and Davies 2004; Dillman et al. 2012).

1.6. OUTLINE OF THIS STUDY

This thesis is divided into 8 chapters.

Chapter 1 is a general introduction, illustrating the context in which this study is situated and explaining the main aims of this work and their logical flow.

Chapters 2-7 contain the effective results, each with their respective specific introduction, material and methods, results and discussion. These chapters have been drafted according to requirements for manuscripts in SCI-indexed journals.

- Chapter 2, **Pilot study on the nematode community during composting**, presents the first detailed data on nematodes during composting.
- Chapter 3, **The nematode fauna in compost**, includes the species description of *Mononchoides composticola* n. sp. isolated from compost, and contains an updated checklist of free-living and plant-parasitic nematodes in Belgium.
- Chapter 4, **The nematode community as a proxy of the microbial community in a rapidly changing compost environment**, describes the temporal succession of both the microbial and the nematode community and their link during a composting process.
- Chapter 5, **Survival and colonization of nematodes in a compost process**, presents the results of experiments on temperature tolerance and on different colonization routes of compost by nematodes, including vectoring by insects.
- Chapter 6, **Factors influencing the nematode community during composting**, describes the relation of the nematode community dynamics with potentially important variables, i.e. temperature, time of composting and the microbial community.
- Chapter 7, **Nematode communities and macronutrients in composts and compost-amended soils as affected by feedstock composition**, presents the short term effect of compost and its associated nematodes on the soil chemical properties and the existing nematode community.

Chapter 8 contains the general discussion, which integrates the principal findings of Chapters 2-7.

1.7. REFERENCES

Achouri, M.S., Medini-Bouaziz, L., Hamaied, S., Charfi-Cheikhrouha, F., 2008. Diversity of terrestrial isopods at the Oued Laou region (Northeast of Morocco): preliminary results. In: Bayed, A., Ater, M. (Eds.) Du Bassin Versant Vers la Mer: Analyse Multidisciplinaire Pour un Gestion Durable. Traveaux de l'Institut Scientifique, Rabat, pp. 75-79.

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresour. Technol. 74, 35-47.

Al-Amidi, A.H.K., 1995. Occurrence of insects and mites in mushroom compost in Ireland. In: Elliott, T.J. (Ed.) 14th International Congress on the Science and Cultivation of Edible Fungi. Science and Cultivation of Edible Fungi, Oxford, 17-22 September, A.A. Balkema Publishers, Rotterdam, pp. 539-546.

Anderson, R.V., 1983. An emended description of *Ditylenchus valveus* Thorne & Malek, 1968 and description of *D. filimus* n. sp. (Nematoda: Tylenchidae) from mushroom compost in Canada. Can. J. Zool. 61, 2319-2323.

Andrassy, I., 1983. A Taxonomic Review of the Suborder Rhabditina (Nematoda: Secernentia), L'office de La Recherche Scientifique et technique outremer, Paris, France, 242 pp.

Bailey, K.L., Lazarovits, G., 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil Till. Res. 72, 169-180.

Baldwin, K.R., Greenfield, J.K., 2009. Composting on Organic Farms, North Carolina Cooperative Extension Service, NC State University, 21 pp.

Barbercheck, M.E., Neher, D.A., Anas, O., El-Allaf, S.M., Weicht, T.R., 2009. Response of soil invertebrates to disturbance across three resource regions in North Carolina. Environ. Monit. Assess. 152, 283-298.

Baranowski, R., 1978. Skalbaggar I frökompost (Coleoptera). Entomol. Tidskr. 99, 37-39.

Baretta, D., Ferreira, C.S., Sousa, J.P., Cardoso, E.J.B.N., 2008. Springtails (Hexapoda: Collembola) as soil quality bioindicators in areas with *Araucaria angustifolia*. Rev. Bras. Cienc. Solo 32, 2693-2699.

Bedano, J.C., Ruf, A., 2010. Sensitivity of different taxonomic levels of soil Gamasina to land use and anthropogenic disturbances. Agric. Forest Entomol. 12, 203-212.

Bergersen, R., Olsen, K.M., Djursvoll, P., Nilssen, A.C., 2006. Centipedes (Chilopoda) and millepedes (Diplopoda) in North Norway. Norw. J. Entomol. 53, 23-39.

Boag, B., Yeates, G.W., 1998. Soil nematode biodiversity in terrestrial ecosystems. Biodivers. Conserv. 7, 617-630.

Bolger, T., 2001. The functional value of species biodiversity - a review. P. Roy. Irish Acad. 101b, 199-204.

Bongers, T., 1990. The maturity index-an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10, 239-251.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. 14, 224-228.

Brimecombe, M.J., De Leij, F.A.A.M., Lynch, J.M., 2001. Nematode community structure as a sensitive indicator of microbial perturbations induced by a genetically modified *Pseudomonas fluorescens* strain. Biol. Fert. Soils 34, 270-275.

Brussaard, L., Kuyper, T.W., Didden, W.A.M., De Goede, R., Bloem, J., 2004. Biological soil quality from biomass to biodiversity - importance and resilience to management stress and disturbance. In: Schjonning, P., Elmholt, S., Christensen, B.T. (Eds.), Managing Soil Quality: Challenges in Modern Agriculture. CAB International, Oxfordshire, pp. 139-161.

Bulluck, L.R., Barker, K.R., Ristaino, J.B., 2002. Influences of organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomatoes. Appl. Soil Ecol. 21, 233-250.

Buddle, C.M., 2010. Photographic key to the pseudoscorpions of Canada and the adjacent USA. Can. J. Arthropod Identif. 10, 1-77.

Chagnon, M., Pare, D., Hebert, C., 2000. Relationships between soil chemistry, microbial biomass and the collembolan fauna of southern Quebec sugar maple stands. Ecoscience 7, 307-316

Clift, A.D., Terras, M.A., 1995. Mites as indicators of compost conditioning. In: Elliott, T.J. (Ed.) 14th International Congress on the Science and Cultivation of Edible Fungi. Science and Cultivation of Edible Fungi, Oxford, 17-22 September, A.A. Balkema Publishers, Rotterdam, pp 507-513

Cogger, C.G., 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Sci. Util. 13, 243-251.

Coleman, D.C., Crossley, D.A., Hendrix, P.F., 2004. Secondary production: activities of heterotrophic organisms- The soil fauna. In: Cella, D. (Ed.) Fundamentals of soil ecology, Elsevier Academic Press, London, UK, pp. 79-185.

Coleman, D.C., Whitman, W.B., 2005. Linking species richness, biodiversity and ecosystem function in soil systems. Pedobiologia 49, 479-497.

Cooperband, L.R., 2000. Composting: Art and science of organic waste conservation to a valuable soil resource. Lab. Med. 31, 283-290.

Crouau, Y., Gisclard, C., Perotti, P., 2002. The use of *Folsomia candida* (Collembola, Isotomidae) in bioassays of waste. Appl. Soil Ecol. 19, 65-70.

Čerevková, A., Renčo, M., 2009. Soil nematode community changes associated with windfall and wildfire in forest soil at the High Tatras National Park, Slovak Republic. Helminthologia 46, 123-130.

D'Hose, T., Cougnon, M., De Vliegher, A., Willekens, K., Van Bockstaele, E., Reheul, D., 2012. Farm compost application: effects on crop performance. Compost Sci. Util. 20, 49-56.

De Cauwer, B., D'Hose, T., Cougnon, M., Leroy, B., Bulcke, R., Reheul, D., 2011. Impact of the quality of organic amendments on size and composition of the weed seed bank. Weed Res. 51, 250-260.

De Cauwer, B., Van den Berge, K., Cougnon, M., Bulcke, R., Reheul, D., 2010. Weed seedbank responses to 12 years of applications of composts, animal slurries or mineral fertilisers. Weed Res. 50, 425-435.

Devliegher, W., Verstraete, W., 1995. *Lumbricus terrestris* in a soil core experiment; Nutrient-Enrichment Processes (NEP) and Gut-Associated Processes (GAP) and their effect on microbial biomass and microbial activity. Soil Biol. Biochem. 27, 573-580.

Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. PLoS Pathog. 8, 1-4.

Dinel, H., Schnitzer, M., Dumonet, S., 1996. Compost maturity: Chemical characteristics of extractable lipids. Compost Sci. Util. 4, 16-25.

Dominguez, J., Edwards, C.A., Ashby, J., 2001. The biology and population dynamics of *Eudrilus eugeniae* (Kinberg) (Oligochaeta) in cattle waste solids. Pedobiologia 45, 341-353.

Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl. Soil Ecol.15, 3-11.

Eiland, F., Klamer, M., Lind, A.M., Leth, M., Baath, E., 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microbial Ecol. 41, 272-280.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Fiera, C., 2009. Biodiversity of Collembola in urban soils and their use as bio-indicators for pollution. Pesqui. Agropecu. Bras. 44, 868-873.

Fjelberg, A., 1998. The Collembola of Fennoscandia and Denmark (Part I) Poduromorpha, Fauna Entomologica Scandinavica Vol. 35, Brill, Leiden, 185 pp.

Forge, T.A., Hogue, E., Neilsen, G., Neilsen, D., 2003. Effects of organic mulches on soil microfauna in the root zone of apple: implications for nutrient fluxes and functional diversity of the soil food web. Appl. Soil Ecol. 22, 39-54.

Gagarin, V.G., 2000. Nematode fauna of manure and compost in Yaroslavl oblast (Russia). Zool. Zh. 79, 1260-1274.

Gamliel, A., Austerweil, M., Kritzman, G., 2000. Non-chemical approach to soilborne pest management—organic amendments. Crop. Prot. 19, 847-853.

Geissen, V., Kampichler, C., 2004. Limits to the bioindication potential of Collembola in environmental impact analysis: A case study of forest soil-liming and fertilization. Biol. Fert. Soils 39, 383-390.

Giere, O., 2009. Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments. Springer-Verlag, Berlin-Heidelberg.

Gisin, H., 1960. Collembolenfauna Europas, Museum d'Histoire Naturelle, Geneva, 312 pp.

Gómez, R.B., Lima, F.V., Ferrer, A.S., 2006. The use of respiration indices in the composting process: A review. Waste Manage. Res. 24, 37-47.

Goralczyk, K., 1998. Nematodes in a coastal dune succession: indicators of soil properties? Appl. Soil Ecol. 9, 465-469.

Goulson, D., Hughes, W.O.H., Chapman, J.W., 1999. Fly populations associated with landfill and composting sites used for household refuse disposal. B. Entomol. Res. 89, 493-498.

Greenslade, P., 2007. The potential of Collembola to act as indicators of landscape stress in Australia. Aust. J. Exp. Agr. 47, 424-434.

Gulvik, M.E., 2007. Mites (Acari) as indicators of soil biodiversity and land use monitoring: A review. Pol. J. Ecol. 55, 415-440.

Gutiérrez, G.P.A., Mazo, K.I.M., 2004. Mesofauna de los Colémbolos en el compost de la corporación univeristaria lasallista. Rev. Lasallista Invest. 1, 102-104.

Hatamoto, M., Tanahashi, T., Murase, J., Matsuya, K., Hayashi, M., Kimura, M., Asakawa, S., 2008. Eukaryotic communities associated with the decomposition of rice straw compost in a Japanese rice paddy field estimated by DGGE analysis. Biol. Fert. Soils 44, 527-532.

Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers - effects on nutrient cycling and plant-growth. Ecol. Monogr. 55, 119-140.

Ishii, K., M. Fukui, Takii, S., 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J. Appl. Microbiol. 89: 768-777.

Kapanen, A., Itavaara, M., 2001. Ecotoxicity tests for compost applications. Ecotox. Environ. Safe. 49, 1-16.

Koehler, H.H., 1999. Predatory mites (Gamasina, Mesostigmata). Agric. Ecosyst. Environ. 74, 395-410.

Kuo, S., Ortiz-Escobar, M.E., Hue, N.V., Hummel, R.L., 2004. Composting and compost utilization for agronomic and container crops. Recent Res. Dev. Environ. Biol. 1, 451-513.

Kutzner, H. J., 2000. Microbiology of composting. In: Rehm, H. J., Reed, G. (Eds.), Biotechnology. Wiley-VCH, pp. 35-100.

Lewis, 1981. The Biology of Centipedes, Cambridge University Press, Cambridge, 476 pp.

López, R., Cabrera, F., Madejón, E., Sancho, F., Álvares, J.M., 2008. Urban composts as an alternative for peat in forestry nursery media. In: Hao, X. (Ed.) Compost I. Dynamic Soil, Dynamic Plant 2 (Special Issue 1), 60-66.

Lundberg, S., Persson, K., 1973. Skalbaggsfaunaen i komposthögar I Lulea. Entomol. Tidskr. 99, 208-210.

Manna, M.C., Jha, S., Ghosh, P.K., Acharya, C.L., 2003. Comparative efficacy of three epigeic earthworms under different deciduous forest litters decomposition. Bioresource Technol. 88, 197-206.

Manso, F.W., 2004. Possible use of nematodes as bioindicator for the maturity of compost. Master Thesis. Ghent University, Faculty of Science, 47 pp.

Messelink, G.J., Bloemhard, C.M.J., 2007. Woodlice (Isopoda) and millepedes (Diplopoda): control of rare greenhouse pests. Proc. Neth. Entomol. Soc. Meet. 18, 43-49.

Moens, T., Yeates , G.W., De Ley, P., 2004. Use of carbon and energy sources by nematodes. In: Cook, R., Hunt, D.J. (Eds). Proceedings of the Fourth Congress of Nematology, June 2002, Tenerife, Spain. Nematology Monographs and Perspectives 2. Leiden, The Netherlands, Brill, pp. 529-545.

Moral, R., Paredes, C., Bustamante, M.A., Marhuenda-Egea, F., Bernal, M.P., 2009. Utilisation of manure composts by high-value crops: Safety and environmental challenges. Bioresource Technol. 100, 5454-5460.

Mulder, C., Schouten, A.J., Hund-Rinke, K., Breure, A.M., 2005. The use of nematodes in ecological soil classification and assessment concepts. Ecotox. Environ. Safe. 62, 278-289.

Nadler, S.A., Carreno, R.A., Adams, B.J., Kinde, H., Baldwin, J.G., Mundo-Ocampo, M., 2003. Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda: Cephalobina: Panagrolaimoidea), an opportunistic pathogen of horses. Int. J. Parasitol. 33, 1115-1125.

Nahar, M.S., Grewal, P.S., Miller, S.A., Stinner, D., Stinner, B.R., Kleinhenz, M.D., Wszelaki, A., Doohan, D., 2006. Differential effects of raw and composted manure on nematode community, and its indicative value for soil microbial, physical and chemical properties. Appl. Soil Ecol. 34, 140-151.

Naidu, R., Megharaj, M., Owens, G., 2004. Recyclable urban and industrial waste - benefits and problems in agricultural use. In: Schjonning, P., Elmholt, S., Christensen, B.T. (Eds.), Managing Soil Quality: Challenges in Modern Agriculture. CAB International, Oxfordshire, pp. 219-237.

Neher, D.A., 2001. Role of nematodes in soil health and their use as indicators. J. Nematol. 33, 161-168.

Neher, D.A., Wu, J., Barbercheck, M.E., Anas, O., 2005. Ecosystem type affects interpretation of soil nematode community measures. Appl. Soil Ecol. 30, 47-64.

Neher, D.A., Darby, B.J., 2009. General community indices that can be used for analysis of nematode assemblages. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.) Nematodes as Environmental Indicators, CAB International, Oxfordshire, pp 107-123.

O'Neill, K.P., Godwin, H.W., Jiménez-Esquilín, A.E., Battigelli, J.P., 2010. Reducing the dimensionality of soil micro invertebrate community datasets using Indicator Species Analysis: Implications for ecosystem monitoring and soil management. Soil Biol. Biochem. 42, 145-154.

Ødegaard, F., Tømmerås, B.A., 2000. Compost heaps - refuges and stepping stones for alien arthropod species in northern Europe. Diversity Distrib. 6, 45-59.

Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments - a review. Appl. Soil Ecol. 44, 101-115.

Palm, T., 1979. Om skalbaggsfaunaen i compost-höger vid Uppsala. Entomol. Tidskr. 100, 33-36.

Pfotzer, G.H., Schüler, C., 1997. Effects of different compost amendments on soil biotic and faunal feeding activity in an organic farming system. Biol. Agric. Hortic. 15, 177-183.

Renčo, M., Sasanelli, N., D'Addabbo, T., Papajova, I., 2010. Soil nematode community changes associated with compost amendments. Nematology 12, 681-692.

Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, S., Bar, A., Orion, D., 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop. Prot. 21, 583-587.

Riedel, P., Navrátil, M., Tuff, I.H., Tufová, J., 2009. Terrestrial isopods (Isopoda: Oniscidae) and millipedes (Diplopoda) of the City of Olomouc (Czech Republic). In: Tajovský, K., Schlaghamerský, J., Pizl, V. (Eds.) Contributions to Soil Zoology in Central Europe III, Bud jovice, The Czech Republic, pp 125-132.

Rosenberg, J., Seifert, G., 1977. Coxal glands of Geophilomorpha (Chilopoda) - Organs of osmoregulation. Cell Tissue Res. 182, 247-251.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J., 2003a. Microbiological aspects of biowaste during composting in a monitored compost bin. J. Appl. Microbiol. 94, 127-137.

Ryckeboer, J., Mergaert, Vaes, K., Klammer, S., De Clerq, D., J., Coosemans, J., Insam, H., Swings, J., 2003b. A survey of bacteria and fungi occuring during composting and self-heating processes. Ann. Microbiol. 53, 349-410.

Rynk, R., 2003. The art in the science of compost maturity. Compost Sci. Util. 11, 94-95.

Sabatini, M.A., Innocenti, G., Montanari, M., Ganassi, S., 2006. Survival and feeding activity of Protaphorura armata in different composts. Pedobiologia 50, 185-190.

Sánchez-Moreno, S., Smukler, S., Ferris, H., O'Geen, A.T., Jackson, L.E., 2008. Nematode diversity, food web condition, and chemical and physical properties in different soil habitats of an organic farm. Biol. Fert. Soils 44, 727-744.

Sánchez-Moreno, S., Nicola, N.L., Ferris, H., Zalom, F.G., 2009. Effects of agricultural management on nematode-mite assemblages: Soil food web indices as predictors of mite community composition. Appl. Soil Ecol. 41, 107-117.

Sellami, F., Hachicha, S., Chtourou, M., Medhioub, K., Ammar, E., 2008. Maturity assessment of composted olive mill wastes using UV spectra and humification parameters. Bioresource Technol. 99, 6900-6907.

Sharma, S., Pradhan, K., Santosh, S., Vasudevan, P., 2005. Potentiality of earthworms for waste management and in other uses - A review. Am. J. Sci. 1, 4-16.

Sharma, V.K., Canditelli, M., Fortuna, F., Cornacchia, G., 1997. Processing of urban and agro-industrial residues by aerobic composting: Review. Energ. Convers. Manage. 38, 453-478.

Smith, I.M., Lindquist, E.E., Behan-Pelletier, V.M., 1998. Mites (Acari). In: Smith, I.M. (Ed.) Assessment of Species Diversity in the Montane Cordillera Ecozone, Ecological Monitoring and Assessment Network, Burlington. Available online: http://www.naturewatch.ca

Skoracka, A., Skoracki, M., Bloszyk, J., Stachowiak, M., 2002. The structure of dominance and frequency of Mesostigmata (Acari) in compost. In: Tajovsky, K., Balik, V., Pizl, V. (Eds.) Studies on Soil Fauna in Central Europe, Proceedings of the 6th Central European Workshop on Zoology, České Budějovice, Czech Republic, pp 69-77.

Steger, K., Jarvis, A., Smars, S., Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. J. Microbiol. Meth. 55, 371-382.

Steger, K., Eklind, Y., Olsson, J., Sundh, I., 2005. Microbial community growth and utilization of carbon constituents during thermophilic composting at different oxygen levels. Microbial Ecol.

50, 163-171.

Steger, K., Sjogren, A.M., Jarvis, A., Jansson, J.K., Sundh, I., 2007. Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste. J. Appl. Microbiol. 103, 487-498.

Sullivan, D.M., Miller, R.O., 2001. Compost quality attributes, measurements , and variability. In: Stofella, P.J., Kahn, B.A. (Eds.), Compost Utilization in Horticultural Cropping Systems. Lewis Publishers, Boca Raton, Fla., pp. 95-120.

Suthar, S., Singh, S., Dhawan, S., 2008. Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? Ecol. Eng. 32, 99-107.

Suthar, S., Singh, S., 2008. Vermicomposting of domestic waste by using two epigeic earthworms(*Perionyx excavatus* and *Perionyx sansibaricus*). Int. J. Environ. Sci. Technol. 5, 99-106.

Termorshuizen, A.J., Moolenaar, S.W., Veeken, A.H.M., Blok, W.J., 2004. The value of compost. Rev. Environ. Sci. Biotechnol. 3, 343-347.

Timper, P., Davies, K.G., 2004. Biotic interactions. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behaviour. CAB International, Wallingford, UK, pp. 277-307.

Tognetti, C., Mazzarino, M.J., Laos, F., 2007. Improving the quality of municipal organic waste compost. Bioresource Technol. 98, 1067-1076.

Tondoh, J.E., Monin Monin, L., Tiho, S., Csuzdi, C.S., 2007. Can earthworms be used as bio-indicators of land-use perturbations in semi-deciduous forest? Biol. Fert. Soils 43, 585-592.

Tuomela, M., Vikman, M., Hatakka, A., Itavaara, M., 2000. Biodegradation of lignin in a compost environment: a review. Bioresource Technol. 72: 169-183.

Vallad, G.E., Cooperband, L., Goodman, R.M., 2003. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. Physiol. Mol. Plant Pathol. 63, 65-77.

Vargha, B., 1995. Three new tardigrade species from Hungary. Acta Zool. Hung. 41, 301-313.

Wallwork, J.A., 1976. An introduction to the soil fauna. In: Wallwork, J.A. (Ed.) The distribution and diversity of soil fauna, Academic Press, London, pp 9-12.

Wichuk, K.M., McCartney, D., 2010. Compost stability and maturity evaluation- a literature review. Can. J. Civ. Eng. 37, 1505-1523.

Yakushev, A.V., Blagodatsky, S.A., Byzov, B.A., 2009. The effect of earthworms on the physiological state of the microbial community at vermicomposting. Microbiology 78, 510-519.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera—an outline for soil ecologists. J. Nematol. 25, 315-331.

Yeates, G.W., Wardle, D.A., Watson, R.N., 1999. Responses of soil nematode populations, community structure, diversity and temporal variability to agricultural intensification over a seven-year period. Soil Biol. Biochem. 31, 1721-1733.

Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biol. Fertil. Soils 37, 199-210.

Yeates, G.W., Ferris, H., Moens, T., Van der Putten, W.H., 2009. The role of nematodes in ecosystems. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, pp. 1-45.

Young, C-C., Rekha, P.D., Arun, A.B., 2005. What happens during composting. In: Chen, Z.S. (Ed.) Compost Production: A Manual for Asian Farmers, Food and Fertilizer Technology Center, Taipei, Taiwan, pp 8-19.

Zelenev, V.V., Berkelmans, R., van Bruggen, A.H.C., Bongers, T., Semenov, A.M., 2004. Daily changes in bacterial-feeding nematode populations oscillate with similar periods as bacterial populations after a nutrient impulse in soil. Appl. Soil Ecol. 26, 93-106.

Zmora-Nahum, S., Markovitch, O., Tarchitzky, J., Chen, Y.N., 2005. Dissolved organic carbon (DOC) as a parameter of compost maturity. Soil Biol. Biochem. 37, 2109-2116.

Zorpas, A.A., 2009. Compost evaluation and utilization. In: Pereira, C.J., Bolin, J.L. (Eds.), Composting: Processing, Materials and Approaches. Nova Science Publishers Inc., New York, pp. 31-68.

Zucconi, F., de Bertoldi, M., 1987. Specifications for solid waste compost. Biocycle 28, 56-61.

Zvomuya, F., Larney, F.J., DeMaere, P.R., Olson, A.F., 2008. Organic amendment effects on crop productivity and nutrient uptake on reclaimed natural gas wellsites. Nutr. Cycl. Agroecosys. 80, 223-232.

2. Pilot study on the nematode community during composting

Modified from:

Steel H, de la Peña E, Fonderie P, Willkens K, Borgonie G and Bert W (2010). Nematode succession during composting and the potential of the nematode community as an indicator of compost maturity. Pedobiologia 53: 181-190.

2.1. ABSTRACT

One of the key issues in compost research is to assess when the compost has reached a mature stage. The maturity status of the compost determines the quality of the final soil amendment product. The nematode community occurring in a Controlled Microbial Composting (CMC) process was analyzed with the objective of assessing whether the species composition could be used as a bio-indicator of the compost maturity status. The results obtained here describe the major shifts in species composition that occur during the composting process. Compared to terrestrial ecosystems, nematode succession in compost differs mainly in the absence of K-strategists and numerical importance of diplogastrids. At the beginning of the composting process (thermophilic phase), immediately after the heat peak, the nematode population is primarily built by bacterial feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogastridae) followed by the bacterial-feeding general opportunists (cp-2) (Cephalobidae) and the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, during the cooling and maturation stage, the bacterial-feeding/predator opportunistic nematodes (*Mononchoides* sp.) became dominant. Finally, at the most mature stage, the fungal-feeding Anguinidae (mainly *Ditylenchus filimus*) were most present. Both, the Maturity Index (MI) and the fungivorous/bacterivorous ratio (f/b ratio), increase as the compost becomes more mature (ranging, respectively, from 1 to 1.86 and from 0 to 11.90). Based on these results, both indices are suggested as potential suitable tools to assess compost maturity.

2.2. INTRODUCTION

Composting is an aerobic, heat-producing and controlled process by which microorganisms convert a mixed organic substrate into carbon dioxide (CO_2), water, inorganic nutrients and stabilized organic matter. Control of the environmental conditions (moisture, temperature, substrate composition and oxygenation) during the process distinguishes composting from natural rotting or decomposition (Zucconi and de Bertoldi 1987; Baldwin and Greenfield 2006). The application of compost to soil has several benefits. The stabilized organic matter in compost improves soil structure. Consequently, soil aeration, soil porosity, water holding capacity and drainage increase (Kuo et al. 2004; Cogger 2005; Baldwin and Greenfield 2006). Furthermore, compost can provide an important source of nutrients for plants (for reviews see Cogger 2005; Zvomuya et al. 2008), especially the compost nitrogen that becomes available for plants after mineralization in the soil (Hadas and Portnoy 1997). In addition, composts are known to

suppress plant diseases through a combination of physiochemical and biological mechanisms (Akhtar and Malik 2000; Gamliel et al. 2000; Reuveni et al. 2002; Bailey and Lazarovits 2003; Vallad et al. 2003; Kuo et al. 2004).

Controlled Microbial Compost (commonly known as CMC or ''Leubke compost'') is a premium grade and well-humified compost. The CMC method was developed through on-farm and laboratory research by the Leubke family (Diver 2004). The composting is carried out in windrows and during the process abiotic parameters such as CO_2 and temperature are intensively monitored. A microbial starter is usually added to inoculate the compost (Diver 2004). This method ensures process control by appropriate aerating through turning, prevention of nutrient leaching and/or reduction of gaseous emissions and heat losses through covering the heap (Rees 2007).

During composting, organic materials are disintegrated by processes associated with several organisms. The taxa that are most important to the composting process are Bacteria, Algae, Fungi, Isopoda, Acari, Nematoda and Protozoa. This wide spectrum of organisms forms a complex and rapidly changing community. Up to the present, only the dynamics of the bacterial community have been thoroughly investigated in relation to composting processes (e.g. Herrmann and Shann 1997; Ishii et al. 2000; Alfreider et al. 2002; Tiquia et al. 2002; Ryckeboer et al. 2003; Tiquia 2005; Fracchia et al. 2006; Halet et al. 2006). Research concerning the role of nematodes in decomposition processes tends to focus on specific natural processes in the soil (Ingham et al. 1985; Ruess 2003; Ruess and Ferris 2004; Wang et al. 2004; Georgieva et al. 2005; Postma-Blaauw et al. 2005) or on laboratory microcosm experiments with bacterial-feeding nematodes and their influence on the decomposition rate of organic material of marine environments (De Mesel et al. 2003, 2004, 2006). Although nematodes appear to be numerically important in the composting process, and their diversity and density in mature compost are considered crucial to constructing a robust soil food web (Ingham 2001, 2006; Ingham and Slaughter 2004), knowledge of the diversity and succession of the nematode community during the composting process is completely lacking. Moreover, nematodes show several characteristics which make them ideal bio-indicators of the ecosystem quality. Nematode community analyses are very useful in assessing ecosystem status and functions since nematodes are ubiquitous and easy to sample (Bongers and Ferris 1999; Ritz and Trudgill 1999; Yeates and Bongers 1999; Neher 2001; Yeates 2003). Furthermore, the species composition of the samples reflects a wide range of abiotic parameters such as substrate texture, climate, biogeography, organic inputs and both natural and anthropogenic disturbances (Yeates 1984; Neher 2001; Yeates 2003). The use of nematodes as functional indicators relies on the allocation

of nematodes to feeding groups (Yeates et al. 1993) and reproductive strategies (cp values) (Bongers 1990; Yeates 2003). Nematode genera with the same cp value are adapted to specific environmental conditions and food sources through anatomical and physiological commonalities (Ferris et al. 2001) and are similar in their responses to disturbance (Bongers 1999; Bongers and Ferris 1999).

One of the key issues in compost research is to assess when the compost has reached a mature stage. The maturity status of the compost determines ultimately the quality of the product. So far numerous tests, based on both physical and chemical parameters, have been proposed (e.g. Butler et al. 2001; Tiquia 2005; Chikae et al. 2006; Castaldi et al. 2008) but unfortunately, many of these have not been proven rigorous, reliable or consistent enough to be used in standard protocols, and those that are would require such a substantial investment in laboratory equipment and staff training that their use in regular composting laboratories is not feasible (Kuo et al. 2004; Baldwin and Greenfield 2006).

Since information on nematode populations in compost is virtually lacking, the first objective of this study was to analyze the structure and succession of the nematode community during a composting process. Secondly, we explored the possibilities of relating the observed structure of the nematode community structure to compost maturity.

2.3. MATERIALS AND METHODS

2.3.1. Study site and sampling

The examined compost heap was located at the Institute for Agricultural and Fisheries Research in Merelbeke, Belgium (Plant Science Unit, Growth and Development research area). The heap was composed of three different feedstock materials: 43% on a volume to volume base (%, v/v) fine wood chips, 43% (v/v) dry hay and 14% (v/v) fresh grass. The C/N ratios of de the feedstock materials were respectively approximately 90/1, 30/1 and 15/1. The heap was 50 m long, 3 m wide and 1.5 m high, and consisted of 3 m^3 feedstock materials per lineal meter. The compost was prepared according to the CMC method (Diver 2004), but no microbial starter was added.

A total of 45 composite samples on 15 different sampling moments were taken during the entire composting process. The entire monitored process lasted six months, from September 2006 to March 2007. During the first three weeks of the process, samples were taken twice a week (6 samples), after which sampling was reduced to once a week for seven weeks (7 samples). At the end of the process the heap was sampled once after one month and again after two months. By following this sampling pattern the complete process was observed and the changes in species

composition throughout could be investigated. Each sample was composed of 20 randomly picked samples of 50 ml of compost each. The samples were mixed to make a total volume of 1 L, from which a subsample of 100 ml was taken for nematode extraction. This sampling procedure was repeated 3 times per sampling event. Hence, replicate samples were taken from a single compost heap and are thus essentially pseudoreplicates.

2.3.2. Data collection: environmental variables

The following abiotic variables were measured at every sampling: temperature (°C), moisture content, pH and percent carbon dioxide ($CO_{2)}$. Temperature and CO_2 content were measured in 3 locations on the heap using specialized equipment (respectively, Digital Thermometer GTH 1150 and Brigon Messtechnik D-63110 Rodgau). For the pH measurements, extractions of 20 g compost in 100 ml distilled water were made. The extractions were shaken by hand 3 times every 2 h and pH was measured with standard electrodes (Consort P400). The moisture content was determined by weighing 50 ml of compost before and after incubation for 48 h at 102 °C.

2.3.3. Nematode community analyses

The existing nematodes in the subsample (100 ml) were extracted from the compost using a modified Baermann funnel method (tray 49 cm x 37.5 cm, basket 38.5 cm x 19.5 cm) (Hooper 1986). Nematodes were counted and 100 individuals were randomly picked out using a stereomicroscope (Leica MZ95). For light microscopical observations, half of the specimens were collected in a very small drop of water in an embryo dish. Formaldehyde (4% with 1% glycerol) was heated to 70 °C and an excess (4-5 ml) was quickly added to the specimens to fix and kill the nematodes (Seinhorst 1966). The fixed nematodes were processed to anhydrous glycerol following the glycerol-ethanol method (Seinhorst 1959, as modified by De Grisse 1969) and mounted on aluminium slides with double cover slips (Cobb 1917). As a supplement to this standard evaluation method, the remaining nematodes were mounted on mass slides (slide 40 mm x 76 mm; cover glass 34 mm x 60 mm). Measurements were prepared manually with a camera lucida on an Olympus BX 51 DIC microscope (Olympus Optical, Tokyo, Japan), which was equipped with an Olympus C5060WZ camera for photographs. Nematodes were identified to genus and species whenever possible. The abundance (individuals/gram dry weight compost) of each genus or species in each sample was determined. Dauer larvae were not included in the total counts and species analysis because accurate identification is often impossible and there immobility hampers a quantitative estimation using a mobility-based nematode extraction. In order to test whether dauer stages or eggs were able to survive the heat peak, additional samples from day 3, 6 and 9 were incubated on agar plates (1% nutrient agar plates containing

2.7 g bacteriological agar, 1.3 g nutrient agar and 80 ml cholesterol (5 mg ml $^{-1}$) in 400 ml medium and 1% bacteriological agar plates containing cholesterol (80 ml 5 mg ml $^{-1}$ in 400 ml medium)). These plate incubations could facilitate reactivation of survival stages possibly present in the samples taken during the heat peak. The resulting data were also not further incorporated in the analysis since incubation experiments impede quantitative analysis and results were not available for all replicates.

Nematode genera were assigned to the 1-5 ''coloniser-persister'' cp scale according to their r and K life-strategy characteristics (Bongers 1990, 1999) and were classified according to their feeding type. These allocations were, respectively, used to calculate the Maturity Index (MI) (Bongers 1990, 1999) and the Trophic diversity Index (TI) (Heip et al. 1985). Additionally, the Structure Index (SI), Enrichment Index (EI) (Ferris et al. 2001) and the fungivorous/bacterivorous ratio (f/b ratio), also providing an indication of the ecosystem condition, were determined. The calculation of the SI and EI is based on guilds, which combine feeding type and cp value to cluster nematode taxa. The EI is based on the expected reaction of non-herbivore, opportunistic nematodes to the increase in food and gives the abundance and activity of primary detritus-feeding nematodes. The SI indicates the sensitivity to disturbances. The EI and SI can be used to construct a faunal profile, which indicates whether the nematode community is basal, enriched or structured (Ferris et al. 2001). The f/b ratio was used as an indicator of the dominant decomposition pathway (Ferris et al. 2001; Ruess 2003; Ruess and Ferris 2004). The Shannon-Wiener index (H'), with log base 2, and the Simpson index (λ) were calculated to measure diversity. The Shannon-Wiener index is more appropriate for rare taxa (Heip et al. 1998) while the Simpson index gives more weight to the predominant taxa (Neher 2001).

2.3.4. Statistical analyses

First, a correlation matrix was performed to unravel possible colinearity among abiotic variables. Subsequently, in order to reduce the redundancy between the different abiotic factors measured during the composting process, a Principal Component Analysis (PCA) was performed, by which the different abiotic variables were related to each other in a single principal component. Due to strong linear dependency in the data set, differences in the MI and f/b ratio during the composting process were addressed by categorizing the data into the different composting phases (thermophilic, cooling and maturing) and performing pair-wise Mann-Whitney tests using composting phase as a factor. All analyses were conducted with the statistical software Statistica 7.0.

2.4. RESULTS

2.4.1. Environmental variables

The abiotic conditions of the compost heap changed dramatically during the composting process (Figure 2.1). The temperature showed two distinct heat peaks; one on day 3 with an average temperature of 71 °C and another on day 15 with an average temperature of 65 °C. After the second peak, the temperature gradually decreased until it reached approximately 10 °C (Figure 2.1A). Although the moisture content and the pH of the compost heap fluctuated during the first 25 days of the process, both parameters stabilized after reaching their maxima (38% and 8.67%, respectively) (Figure 2.1B and C). The CO_2 concentration (Figure 2.1D) also showed two maxima during the first 29 days (15% on day 18, and 18% on day 6) and decreased afterwards in two stages (from 4.5% to 0%). Correlation analysis revealed that temperature was positively correlated with CO_2 concentration, moisture content and ambient temperature (Table 2.1). Ambient temperature also correlated positively with CO_2 concentration and negatively with both moisture content and pH. The PCA performed using heap temperature, pH, CO_2 and humidity reduced the variation to only one principal component which accounted for 67% of the variation within the data matrix and to which heap temperature and humidity contributed the most (see vector loadings, Table 2.1).

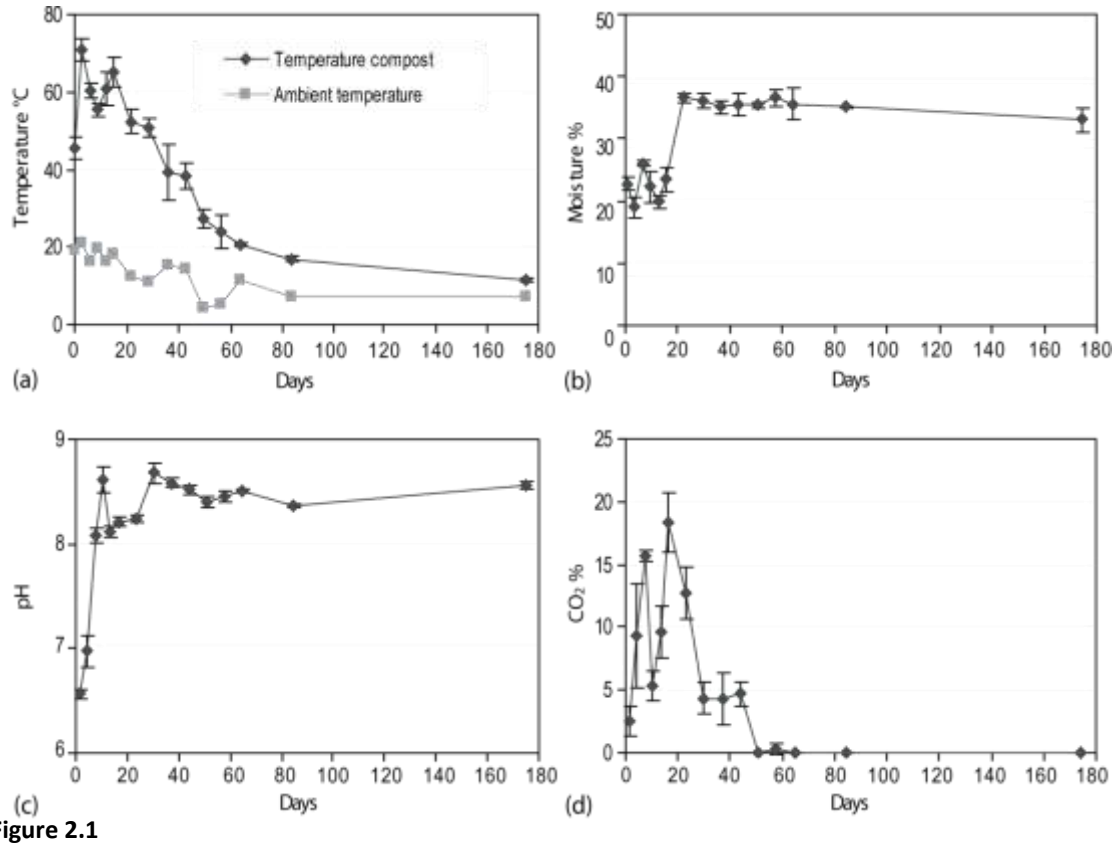


Figure 2.1

Environmental variables measured during the composting process including (a) temperature of the compost (°C) and the ambient temperature (°C), (b) moisture content (%), (c) pH values and (d) CO_2 concentration (%). Error bars indicate SD.

Table 2.1
Pearson correlation coefficients and product moment correlation coefficients among continuous environmental variables (Temperature, CO_2, Moisture, pH, ambient temperature) measured during the composting process and the vector loadings of the Principal Component Analysis (PCA).

	Temperature	CO_2	Moisture content	pH	Env. Temp.	Vector Loadings PCA
Temperature	1					-0.91
CO_2	0.83^{***}	1				-0.76
Moisture content	-0.67^{**}	-0.5	1			0.86
pH	-0.43	-0.20^{*}	0.62^{*}	1		0.64
Ambient Temp.	0.86^{***}	0.61^{*}	-0.73^{**}	-0.55^{**}	1	-

(*p<0.05; **p<0.01; ***p≤0.001)

2.4.2. Nematode community analyses

Species composition and number of nematodes

In total 18 genera belonging to 12 families were identified from the 15 sampling occasions during the complete process. The individual samples were generally characterized by relatively few taxa (maximum 9 different species). The most common taxa were *Aphelenchoides* sp., *Diploscapter coronatus*, *Mononchoides* sp., *Cephalobus* sp. and *Panagrolaimus labiatus*. Other taxa, e.g. *Diplogastrellus* sp., *Diplogaster* sp., *Halicephalobus gingivalis* and *Ditylenchus filimus*, were rare and were detected only at very specific periods during the process. Table 2.2 provides an overview of the species detected at every sampling occasion. For more detailed information about the species observed during the composting process, see http://www.nematology.ugent.be/vce.html. Also survival stages could be isolated with the modified Baermann funnel method (Table 2.2). The number of nematodes gradually increased with decrease in temperature (Figure 2.2A). Immediately after the heat peak, on day 3 until day 9, nematodes could not be detected using the Baermann funnel method. However, incubation of these compost samples on agar plates provided cultures of *H. gingivalis* and *D. coronatus* (Table 2.2). Most likely these were reactivated dauer stages or temperature resistant eggs. Thereafter, the number of nematodes increased gradually until the final sampling event. The highest numbers of nematodes were found in the first and in the last samples (respectively, 1234 and 933 nematodes per 100 g dry weight compost).

Table 2.2

Nematode species list with presence of the species during the composting process and information on the dauer larvae found during the process. The feeding type of each species is also provided (3=bacterial-feeding, 2=fungal-feeding, 3-5a=bacterial-feeding/predator, 8=omnivorous). All species were isolated using the modified Baermann funnel method except for the species with an asterisk, which were isolated after incubation of compost on agar plates.

TAXA	Feeding-type	Day														
		0	3	6	9	12	15	22	29	36	43	50	57	64	84	175
Rhabditis sp.1	3	■														
Rhabditis sp.2	3	■														
Rhabditis sp.3	3	■														
Diplogastrellus sp.	3	■														
Diplogasteritus sp.	3	■					■		■			■	■	■		■
Panagrolaimus labiatus	3	■					■	■			■	■	■			
Rhabditidae sp.	3						■	■		■	■	■	■	■	■	■
Cephalobidae sp.	3							■								
Cephalobus sp.1	3							■	■	■	■	■	■	■	■	
New genus Ordo Rhabditina	3							■	■	■			■			
Diploscapter coronatus	3									■		■	■	■	■	■
Cephaloboides sp.1	3													■		
Poikilolaimus sp.1	3													■	■	■
Halicephalobus gingivalis	3														■	
Rhabditophanes cobbi	3															■
Mesorhabditis sp.1	3															
Aphelenchoididae sp.1	2	■							■	■		■	■	■	■	
Aphelenchoides sp.1	2							■	■	■	■	■	■	■	■	
Aphelenchoides sp.2	2										■					
Paratylenchus sp.	2													■		
Ditylenchus filimus	2														■	■
Diplogaster sp.	3-5a	■														
Mononchoides sp.	3-5a								■	■	■	■	■	■	■	■
Qudsianematidae sp.	8													■		
SURVIVAL STAGE RECORD																
Rhabditidae dauer											■					
Diplogastridae dauer								■	■	■		■	■	■	■	
Unknown dauer										■						
*Halicephalobus gingivalis**			■	■	■											
*Diploscapter coronatus**																

Functional groups: feeding type and cp value

The composition of nematode feeding types showed a clear dominance of bacterial feeders (16 species or 55% of the total abundance), followed by the subdominant fungal-feeders (5 species or 33.3% of the total abundance) and the bacterial-feeding and predatory (of nematodes) nematodes (2 species or 11.7% of the total abundance) (Table 2.3). Figure 2.2B displays the feeding-type compositions per sample based on the 3 replicas. Bacterial-feeding nematodes were present in all nematode-containing samples, but most samples included fungal-feeders and bacterial-feeding/predator nematodes as well as bacterial-feeders (days 36, 43, 50, 57, 64, 84 and 175). For the first 50 days of the process, bacterial-feeding nematodes were clearly dominant. From day 22 onwards, the fungal-feeding nematodes were also detected in the

samples. The density of the bacterial-feeding/predator nematodes increased gradually from day 29 onwards, and this type dominated the samples of days 64 and 84. In the most mature sample the fungal-feeders were clearly dominant.

The distribution of the nematode genera from the composting process along the cp scale was uneven. The enrichment opportunists (cp-1 value) were dominant (16 species or 66.7% of the total abundance) while the general opportunists (cp-2 value) were subdominant (7 species or 33.3% of the total abundance). One genus with a cp-4 value was present on day 64 but genera with a cp-3 value were completely absent (Table 2.3). The most mature stage (day 175) was dominated by cp-2 genera, unlike the other samples, wherein the genera with a cp-1 value were dominant (Table 2.3).

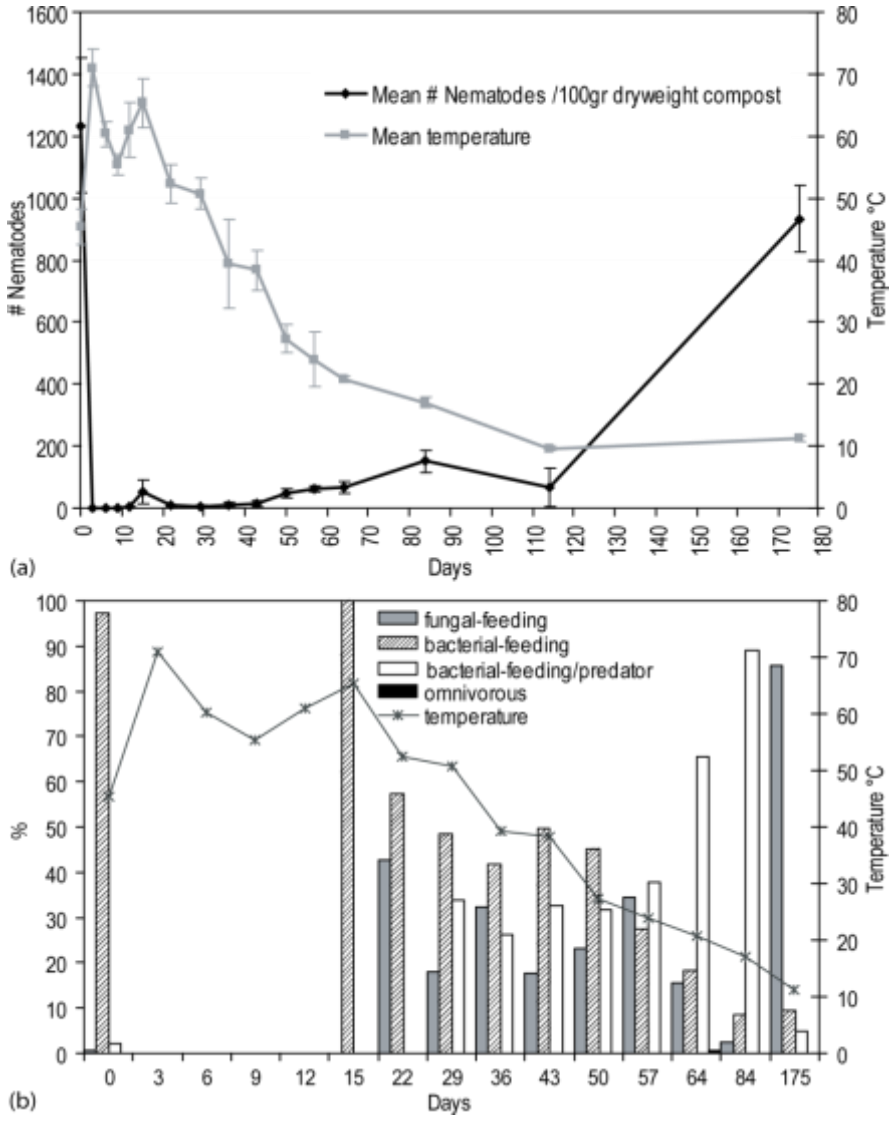


Figure 2.2

(a) The number of nematodes and the temperature of the compost during the process. (b) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator, omnivorous nematodes) and the temperature of the compost at every sampling occasion.

Indices and integration of the abiotic data

At the beginning of the process and immediately after the heat peak, the TI, based on the defined feeding types, was relatively high, due to the dominance of a single feeding type (i.e. bacterial-feeding) (Table 2.3). The lowest TI values, due to the more equal distribution of the feeding types in the samples, appear from day 29 onwards, in the first half of the composting process when the temperature is still relatively high (40-50 °C). At the most mature stage, when the temperature of the heap most closely approximated the ambient temperature, the TI increased again as a result of the dominance of one feeding group (i.e. fungal-feeding). The f/b ratio showed a distinct change during the process (Table 2.3). The f/b ratio fluctuated between 0 and 2.68 with a maximal peak (11.90 ± 8.15) on day 175, in line with the enhancement of fungal-feeding nematodes (Table 2.3). The cp values of the genera were used to calculate the MI (Table 2.3). For the first 84 days of the process, the MI varied between 0.68 and 1.58 (Table 2.3). The pair-wise Mann-Whitney test revealed differences in the MI and f/b ratios between the different phases of the composting process (thermophilic phase: days 3-29, cooling phase: days 36-64, maturing phase: days 84-175) (Table 2.4). The mean MI and f/b ratio during the three composting phases are given in Table 2.4. The MI and the f/b ratio both showed a significant difference between the maturing and the two previous phases (p values, respectively, 0.001 and 0.05) (Table 2.4). Mark that especially the f/b ratio and maturity index trend are strongly influenced by the results of one single divergent sampling point (day 175). This limitation necessitates caution in the further interpretation of the statistical results. The diversity was highest on days 36 and 57, and slightly lower at the end of the process (Table 2.3). However, the observed fluctuations in diversity were not statistically significant. Neither the EI nor the SI showed a clear trend during the process, nor could they be linked to nematode succession. This could be attributed to the fact that these indices are designed for more complex, larger scale and longer lasting processes.

In summary, the taxonomic analysis of the nematode community revealed three clear successional phases after the heat peak. At the beginning of the process, just after the temperature peak observed on day 3, the nematode population was made up primarily of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogastridae) followed by the bacterial-feeding general opportunists (cp-2) (Cephalobidae) and the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, the opportunistic bacterial-feeding/predator nematodes (*Mononchoides* sp.) became the dominant species. Finally, at the most mature stage, the fungal-feeding Anguinidae (e.g. *D. filimus*) was the most abundant group.

Table 2.4
Mean Maturity Index and f/b ratio during the three different phases of the composting process.

	Composting phase			
Index	**Thermophilic**	**Cooling**	**Maturing**	**P**
MI	0.81±0.59*	1.24±0.12*	1.34±0.34	0.001
f/b	0.30±0.56	1.50±1.72	3.58±6.13*	0.05

Table 2.3

Overview of the means ±SD of the abundance of nematodes (per 100 g DW compost), number of genera, percent contribution to the functional groups (trophic groups: type 2 (fungal-feeding nematodes), type 3 (bacterial-feeding nematodes), type 3-5a (bacterial-feeding/predator nematodes), type 8 (omnivorous nematodes) and cp-groups: cp-1 (enrichment opportunists), cp-2 (general opportunists), cp-4 (persisters)) and the values of the indices: TI (Trophic Index), f/b (fungivorous/bacterivorous ratio), MI (maturity index), EI (enrichment index), SI (structure index), H' (Shannon-Wiener index) and λ (Simpson Index)) at every sampling event.

	DAY														
	0	**3**	**6**	**9**	**12**	**15**	**22**	**29**	**36**	**43**	**50**	**57**	**64**	**84**	**175**
Adundance	1234 ±217.65	0	0	0	0	53 ±37.64	8 ±1.04	6 ±2.35	13 ±7.44	25 ±13.25	49 ±13.43	64 ±9.68	68 ±18.77	151 ±35.09	933 ±106.78
# Genera	4 ±1	0	0	0	0	3 ±1	3 ±1	3 ±2	3 ±1	3 ±1	6 ±1	6±1	7 ±2	5 ±1	5 ±1
Functional groups %															
Trophic groups %															
type 2	1 ± 0.8	0	0	0	0	0	43 ±20.1	18 ±21.7	32 ±14.3	18 ±17.0	23 ±7.5	35 ±19.1	15 ±9.4	3 ±1.5	86 ±7.1
type 3	97 ± 1.7	0	0	0	0	100 ±0.0	57 ±20.1	48 ±38.2	42 ±30.3	50 ±53.0	45 ±12.2	28 ±3.4	18 ±8.6	8 ±2.5	10 ±5.2
type 3-5a	2 ±2.3	0	0	0	0	0	0	34 ±26.0	26 ±34.7	33 ±37.8	32 ±19.6	38 ±17.3	66 ±2.5	89 ±3.9	5 ±1.9
type 8	0	0	0	0	0	0	0	0	0	0	0	0	1 ±0.9	0	0
Cp-groups %															
cp-1	99 ±0.8	0	0	0	0	100 ±0.0	57 ±20.1	82 ±21.7	68 ±14.3	82 ±17.0	77 ±7.5	65 ±19.1	84 ±9.8	97 ±1.5	14 ±7.1
cp-2	1 ±0.8	0	0	0	0	0	43 ±20.1	18 ±21.7	32 ±14.3	18 ±17.0	23 ±7.5	35 ±19.1	15 ±9.4	3 ±1.5	86 ±7.1
cp-4	0	0	0	0	0	0	0	0	0	0	0	0	1 ±1.0	0	0
Indices															
TI	0.94 ± 0.03	0	0	0	0	1.00 ±0.00	0.76 ±0.21	0.61 ±0.34	0.48 ±0.06	0.64 ±0.24	0.40 ±0.07	0.39 ±0.03	0.50 ±0.04	0.80 ±0.06	0.75 ±0.11
f/b	0.00 ± 0.01	0	0	0	0	0	0.83 ±0.63	0.50 ±0.87	1.33 ±1.45	2.68 ±2.50	0.52 ±0.04	1.24 ±0.83	0.92 ±0.74	0.28 ±0.12	11.90 ±8.15
MI	1.00 ± 0.01	0	0	0	0	1.00 ±0.00	1.41 ±0.20	1.13 ±0.22	1.29 ±0.14	1.21 ±0.17	1.24 ±0.08	1.33 ±0.19	1.15 ±0.11	1.03 ±0.01	1.86 ±0.07
EI	100.00 ±0.19	0	0	0	0	100.00 ±0.00	84.94 ±9.58	96.00 ±6.93	91.27 ±4.79	93.45 ±5.56	93.14 ±2.52	98.41 ±7.36	96.18 ±2.86	99.37 ±0.35	62.25 ± 5.39
SI	0	0	0	0	0	0	0	0	0	0	0	0	8.45 ±14.63	0	0
H'	1.21 ±0.34	0	0	0	0	0.85 ±0.19	1.12 ±0.21	0.84 ±0.76	1.05 ±0.19	0.69 ±0.45	1.32 ±0.25	1.38 ±0.10	1.15 ±0.17	0.48 ±0.13	0.57 ±0.22
λ	0.37 ±0.16	0	0	0	0	0.47 ±0.06	0.36 ±0.09	0.55 ±0.40	0.40 ±0.09	0.63 ±0.26	0.33 ±0.08	0.31 ±0.04	0.47 ±0.04	0.79 ±0.07	0.75 ±0.11

2.5. DISCUSSION

The nematode community in compost has hitherto never been thoroughly investigated. The results obtained in this study describe major shifts in species composition during the composting process, and moreover, link these structural community changes with the shifts in abiotic conditions that take place during the process.

Several authors have already proposed the use of nematode assemblages as powerful tools to analyze ecosystem processes and quality (e.g. Bongers and Ferris 1999; Ritz and Trudgill 1999; Ferris et al. 2001; Neher 2001; Ferris and Bongers 2009; Yeates et al. 2009). The calculation of nematode-based indices is based on the allocation to functional groups and basic insights into the successional changes within a given studied system. A composting process is most likely an outstanding example of an ecosystem in transition that can be evaluated by the succession of nematodes.

In our case, the maturity index and the f/b ratio showed a clear pattern during the process and therefore seem to be suitable indices to evaluate the composting process. The MI and the f/b ratio both increase as the compost becomes more mature. According to Bongers (1999), a rapidly changing environment with an abundance of food is typically inhabited by opportunistic nematodes, starting with enrichment opportunists (cp-1), which are gradually replaced by general opportunists (cp-2). This trophic situation results in relatively low MI values at the beginning of the process and, by contrast, significantly higher MI values at the end of the process. The f/b ratio clearly reflects the shift in prevalence of bacterial-feeding nematodes in the thermophilic and cooling phases, to fungal-feeding nematodes in the maturation phase. This ratio can also be used to indicate the dominating decomposing pathway in a decomposing environment (Bongers and Bongers 1998; Ruess 2003; Ruess and Ferris 2004). From our analysis we could see that the first 84 days of the process, during which the f/b ratio is typically relatively low, decomposition occurred mainly through the bacterial-dominating pathway. However, in the final stage of the process (day 175), when the ratio is typically highest, the decomposition mainly occurred through the fungi-dominated pathway. This pattern may be an indication of a retardation of the decomposition rate due to the fungal-associated decomposition of more complex organic materials (Ruess and Ferris 2004).

A more detailed analysis of the successional changes of the nematode community revealed that the composting process actually undergoes a meticulous succession of r-strategists. According to Wharton (1986), habitats subject to environmental extremes do not favor K-strategists. This is most likely the main reason for their absence during the composting process. The first

nematodes capable of colonizing the compost during the last phase of the heat peak (= thermophilic phase, T ≥ 45 °C) belonged to the genera *Diplogasteritus*, *Panagrolaimus* and *Rhabditis*. This first population (on day 15) of early colonizers in a nutrient-rich environment was 100% bacterial-feeding and enrichment opportunistic nematodes, as proposed by Bongers (1990) and confirmed by many others (e.g. Ettema and Bongers 1993; Bardgett et al. 1998; Wasilewska 1998). At the very beginning of the cooling phase, from days 22-29, this first population was subverted by a population dominated by the genera *Cephalobus* and *Aphelenchoides*, both general opportunists (cp-2) and respectively bacterial and fungal-feeding. The replacement of the enrichment opportunists belonging to the Rhabditidae family (cp-1) by general opportunists from the family Cephalobidae (cp-2) and the occurrence of fungal-feeding nematodes has also been observed by Ferris and Matute (2003), Wang et al. (2004) and Georgieva et al. (2005) during the decomposition of several plant residues in soil. During the cooling phase (45 °C- ambient temperatures), occurring from day 36 onwards, the dominance of the former nematodes started to decrease and other species started to inhabit the compost: e.g. *D. coronatus*, *Rhabditis* (*Cephaloboides*) sp. and *Mononchoides* sp. Since bacterial-feeding fauna mirrors previous bacterial production (Georgieva et al. 2005; Ferris and Bongers 2006), this shift in nematode species composition and increase in the number of nematodes can be linked to increase in bacterial activity in the cooling phase and to the presence of completely different bacterial populations in the thermophilic and cooling phases as described by Ishii et al. (2000), Ryckeboer et al. (2003) and Halet et al. (2006). The cooling phase environment (≤ 45 °C), with bacteria, fungi and various nematode species in abundance, gave *Mononchoides* sp. the opportunity to start blooming. This differs from decomposition in soil where Neodiplogasteridae (including *Mononchoides*) dominate from the beginning (Georgieva et al. 2005). This retardation might possibly be explained by the relatively high and lethal temperatures in the beginning of the cooling phase (± 40 °C). During maturation at the end of the process, from days 84-175, a shift in prevalence of bacterivorous to fungivorous nematodes took place, which can be associated with the transition from mainly bacterial activity during the thermophilic and early cooling stages to an increase in activity of fungi in late cooling and maturation stages (Ryckeboer et al. 2003). Moreover, it is known that fungal energy channels predominate when the organic material is of a high C/N ratio and, conversely, bacterial decomposition channels predominate when the organic material is of a low C/N ratio (Ruess 2003; Ruess and Ferris 2004) (cfr. high C/N ratios of feedstock materials). In a study by Ferris and Matute (2003), the rate of succession from bacterivorous to fungivorous nematodes increased in plots receiving high C/N materials (Ferris and Matute 2003). Most likely, when looking at the whole process, during the

thermophilic phase the extreme environmental circumstances (i.e. temperature) are the limiting factors for nematode succession, whereas during the mesophilic and maturation phases, food resources might be the most important selective force for successional events.

According to the scanty literature, remarkably similar taxa to those observed in this study have been described from compost (Gagarin 2000; http://www.soilfoodweb.com). The majority of species described from other compost heaps also belonged to the rhabditids and the diplogasterids and nearly always the same genera (i.e. *Cephalobus*, *Rhabditis*, *Diploscapter*, *Aphelenchoides* and *Ditylenchus*)(Gagarin 2000; http://www. soilfoodw eb.com). More specific similarities with other observations from compost include *P. labiatus* (China; Andrassy 1984), *D. filimus* (West Canada; Anderson 1983), *H. gingivalis* (USA, Riverside; Nadler et al. 2003) and *Rhabditis* (*Poikilolaimus*) sp. (mushroom compost, Russia; Gagarin 2000). Remarkably, notwithstanding the nematode destructive heat peak, the geographic disparity and different feedstock materials, the same genera and even the same species were found in these studies, including this one. Where do these ‘‘compost’’ nematodes come from? The composting process is an ‘‘open’’ process and the ability to arrive in a new habitat does not necessarily mean that the nematodes also become established. As a result it makes little difference whether the species come from ‘‘immigrations’’ by movement of air, water and tillage machinery or from awakened dauer stages or eggs, the surrounding soil or by insect phoresy, because the typical compost environment will ultimately serve as the fundamental selective force regardless of origin (for overview on nematode dispersal see Hodda et al. 2009). Hence, the nematode indices proposed herein for assessing compost maturity might be of universal relevance, although further research is required to clarify the ways in which nematodes can arrive at a compost heap.

Unlike Manso (2004), who only found bacterial-feeding Rhabditidae in mature compost, diplogasterids appeared to be numerically important (4 different genera were found). In particular, *Mononchoides* sp. was numerically important from days 29-84. According to Yeates et al. (1993), 2 of the recorded diplogasterid genera (*Diplogastrellus* and *Diplogasteritus*) are strictly bacterial-feeding nematodes (feeding type 3). The 2 other genera (*Mononchoides* and *Diplogaster*) could belong to the bacterial-feeding nematodes as well as to the predator nematodes (feeding type 5a) and to the omnivorous nematodes (feeding type 8) (Yeates et al. 1993). Because species within the same feeding group may vary in their food resources and some species can feed on several food resources, the allocation of nematodes to a specific feeding type is often uncertain (Yeates et al. 1993; Yeates 2003). The herein numerically very important *Mononchoides* sp. appeared to have a biphasic feeding ability (as described earlier by

Yeates 1969, 1970), namely on bacteria and other nematode species (Chapter 3, part 2). The neodiplogasterids *Mononchoides* sp. and the diplogasterid *Diplogaster* sp. were therefore allocated to a combined feeding group ''bacterial-feeding/predator'' (feeding type 3-5a) (see also Georgieva et al. 2005). However, most terrestrial nematode indices (e.g. EI, CI and TI) do not incorporate this new feeding type and thus are not usable for compost. Diplogasterids are known to be prey-selective to plant-parasitic nematodes (Khan and Kim 2007), and therefore, are very promising biological control agents (Bilgrami and Jairajpuri 1988; Bilgrami et al. 2005 ; Bilgrami 2008). This study shows that a composting process can provide a great range of potential predator nematodes. This is particularly an important factor in the suppressive capacity of compost against plant diseases.

In conclusion, this study produced some promising results. The successional changes of the nematode community during the process demonstrated opportunities to describe and evaluate the condition of the composting process. Although further research needs to be performed in order to strengthen these findings, the nematode-based indices maturity index and fungivorous/bacterivorous ratio are probably the most suitable tools to assess compost maturity. Thus, the next step should be to analyze different composting processes and more time frames in order to correlate particular maturity index and fungivorous/bacterivorous ranges to the state and maturity of the compost process. Finally, further work is required to asses the effectiveness and importance of the remarkably high number of bacterial-feeding/predator nematodes during certain compost stages on the potential suppressive effect of compost.

Acknowledgements

Hanne Steel and Eduardo de la Peña acknowledge the Foundation for Scientific Research, Flanders (FWO-Vlaanderen, Belgium) for providing an aspirant grant and a postdoctoral fellowship, respectively.

2.6 REFERENCES

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technol. 74, 35-47.

Alfreider, A., Peters, S., Tebbe, C.C., Rangger, A., Insam, H., 2002. Microbial community dynamics during composting of organic matter as determined by 16S ribosomal DNA analysis. Compost Sci. Util. 10, 303-312.

Anderson, R.V., 1983. An emended description of *Ditylenchus valveus* Thorne & Malek, 1968 and description of *D. filimus* n. sp. (Nematoda: Tylenchidae) from mushroom compost in Canada. Can. J. Zool. 61, 2319-2323.

Andrassy, I., 1984. In: Bestimmungsbucher zur bodenfauna europas: Klasse Nematoda: (Ordnungen: Monhysterida, Desmoscolecida, Aeraeolaimida, Chromodorida, Rhabditida). Gustav Fischer Verlag, Stuttgart, pp. 228.

Bailey, K.L., Lazarovits, G., 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil Till. Res. 72, 169-180.

Baldwin, K.R., Greenfield, J.K., 2006. Composting on organic farms. Publication no. AG-659W-01. North Carolina Cooperative Extension Service, NC State University.

Bardgett, R.D., Keiller, S., Cook, S., Gilburn, A.S., 1998. Dynamic interactions between soil animals and microorganism in upland grassland soils amended with sheep dung: a microcosm experiment. Soil Biol. Biochem. 30, 531-539.

Beffa, T., Blanc, M., Marilley, L., Lott Fischer, J., Lyon, P.-F., Aragno, M., 1996. Taxonomic and metabolic microbial diversity during composting. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), The Science of Composting . Blackies Academic and Proffessional, Glasgow, Scotland, pp. 149-161.

Bilgrami, A.L., 2008. Biological control potentials of predatory nematodes. In: Ciancio, A., Mukerji, K.G. (Eds.), Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer, pp. 3-28.

Bilgrami, A.L., Jairajpuri, M.S., 1988. Attraction of *Mononchoides longicaudatus* and *M. fortidens* (Nematoda: Diplogasterida) towards prey and factors influencing attraction. Rev. Nématol. 11, 195-202.

Bilgrami, A.L., Gaugler, R., Brey, C., 2005. Prey preference and feeding behavior of the diplogastrid predator *Mononchoides gaugleri* (Nematoda : Diplogastrida). Nematology 7, 333-342.

Bongers, T., 1990. The maturity index-an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Bongers, T., 1999. The maturity index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. Plant Soil 212, 13-22.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10, 239-251.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. 14, 224-228.

Butler, T.A., Sikora, L.J., Steinhilber, P.M., Douglass, L.W., 2001. Compost age and sample storage effects on maturity indicators of biosolids compost. J. Environ. Qual. 30, 2141-2148.

Castaldi, P., Garau, G., Melis, P., 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and watersoluble fractions. Waste Manage. 28, 534-540.

Chikae, M., Ikeda, R., Kerman, K., Morita, Y., Tamiya, E., 2006. Estimation of maturity of compost from food wastes and agro-residues by multiple regression analysis. Bioresour. Technol. 97, 1979-1985.

Cobb, N.A., 1917. Notes on nemas. Contr. Sci. Nematol 5, 117-128.

Cogger, C.G., 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Sci. Util. 13, 243-251.

De Grisse, A.T., 1969. Redescription ou modifications de quelques techniques utilisées dans l' étude des nématodes phytoparasitaires. Meded. Rijksfak. Landbouwwet. Gent 34, 351-369.

De Mesel, I., Derycke, S., Swings, J., Vincx, M., Moens, T., 2003. Influence of bacterivorous nematodes on the decomposition of cordgrass. J. Exp. Mar. Biol. Ecol. 296, 227-242.

De Mesel, I., Derycke, S., Moens, T., Van der Gucht, K., Vincx, M., Swings, J., 2004. Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environ. Microbiol. 6, 733-744.

De Mesel, I., Derycke, S., Swings, J., Vincx, M., Moens, T., 2006. Role of nematodes in decomposition processes: does within-trophic group diversity matter?. Mar. Ecol. Prog. Ser. 321, 157-166.

Diver, S., 2004. Controlled microbial composting and humus management: Leubke compost. ATTRA—National Sustainable Agriculture Information Service. <http://www.ibiblio.org/steved/Leubke/Leubke-compost2.html>.

Ettema, C.H., Bongers, T., 1993. Characterization of nematode colonization and succession in disturbed soil using the Maturity Index. Biol. Fertil. Soils 16, 79-85.

Ferris, H., Bongers, T., 2006. Nematode indicators of organic enrichment. J. Nematol. 38, 3-12.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Ferris, H., Matute, M.M., 2003. Structural and functional succession in the nematode fauna of a soil food web. Appl. Soil Ecol. 23, 93-110.

Ferris, H., Bongers, T., 2009. Indices developed specifically for analysis of nematode assemblages. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, pp. 124-146.

Fracchia, L., Dohrmann, A.B., Martinotti, M.G., Tebbe, C.C., 2006. Bacterial diversity in a finished compost and vermicompost: differences revealed by cultivationindependent analyses of PCR-amplified 16S rRNA genes. Appl. Microbiol. Biotechnol. 71, 942-952.

Gagarin, V.G., 2000. Nematode fauna of manure and compost in Yaroslavl oblast (Russia). Zool. Zh. 79, 1260-1274.

Gamliel, A., Austerweil, M., Kritzman, G., 2000. Non-chemical approach to soilborne pest management—organic amendments. Crop. Prot. 19, 847-853.

Georgieva, S., Christensen, S., Stevnbak, K., 2005. Nematode succession and microfauna-microorganism interactions during root residue decomposition. Soil Biol. Biochem. 37, 1763-1774.

Hadas, A., Portnoy, R., 1997. Rates of decomposition in soil and release of available nitrog en from cattle manure and municipal waste composts. Compost Sci. Util. 5, 48-54.

Halet, D., Boon, N., Verstraete, W., 2006. Community dynamics of methanotrophic bacteria during composting of organic matter. J. Biosci. Bioeng. 101, 297-302.

Heip, H.R.C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Ann. Rev. 23, 399-489.

Heip, H.R.C., Soetaert, P.M.J.H., Soetaert, K., 1998. Indices of diversity and evenness. Océanis 24, 61-67.

Herrmann, R.F., Shann, J.F., 1997. Microbial community changes during composting of municipal solid waste. Microb. Ecol. 33, 78-85.

Hodda, M., Peters, L., Traunspurger, W., 2009. Nematode diversity in terrestrial, freshwater aquatic and marine systems. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, pp. 45-93.

Hooper, D.J., 1986. Extraction of free-living stages from soil. In: Southey, J.F. (Ed.), Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 5-30.

Ingham, E.R., 2001. Micronized compost and microbial live in compost. Biocycle, 58-62.

Ingham, E.R., 2006. Agriculture has a future: even with reduced fertilization and reduced pesticide use. Soil Management Focused on Plant Production and Cattle Breeding with Living Inputs: Compost and Compost-Tea. Symposium FARMcompost ILVO, Merelbeke, Belgium.

Ingham, E.R., Slaughter, M.D., 2004. The soil food web—soil and compost as living ecosystems. First International Conference Soil and Compost Eco-Biology, León, Spain.

Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers—effects on nutrient cycling and plant-growth. Ecol. Monogr. 55, 119-140.

Ishii, K., Fukui, M., Takii, S., 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. J. Appl. Microbiol. 89, 768-777.

Khan, Z., Kim, Y.H., 2007. A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Appl. Soil Ecol. 35, 370-379.

Kuo, S., Ortiz-Escobar, M.E., Hue, N.V., Hummel, R.L., 2004. Composting and compost utilization for agronomic and container crops. Recent Res. Dev. Environ. Biol. 1, 451-513.

Manso, F.W., 2004. Possible use of nematodes as bioindicator for the maturity of compost. Master Thesis. Ghent University, Faculty of Science, pp. 22-31.

Nadler, S.A., Carreno, R.A., Adams, B.J., Kinde, H., Baldwin, J.G., Mundo-Ocampo, M., 2003. Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda: Cephalobina: Panagrolaimoidea), an opportunistic pathogen of horses. Int. J. Parasitol. 33, 1115-1125.

Neher, D.A., 2001. Role of nematodes in soil health and their use as indicators. J. Nematol. 33, 161-168.

Postma-Blaauw, M.B., de Vries, F.T., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2005. Within-trophic group interactions of bacterivorous nematode species and their effects on the bacterial community and nitrogen mineralization. Oecologia 142, 428-439.

Rees, L., 2007. Compost: The microbial way. Bristol, Soil Association Food and Farming Department. <www.soilassocation.org/foodandfarming>.

Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, S., Bar, A., Orion, D., 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop. Prot. 21, 583-587.

Ritz, K., Trudgill, D.L., 1999. Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges—discussion paper. Plant Soil 212, 1-11.

Ruess, L., 2003. Nematode soil faunal analysis of decomposition pathways in different ecosystems. Nematology 5, 179-181.

Ruess, L., Ferris, H., 2004. Decomposition pathways and successional changes. In: Proceeding of the Fourth International Congress of Nematology, vol. 2, pp. 547-556.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J., 2003. Microbiological aspects of biowaste during composting in a monitored compost bin. J. Appl. Microbiol. 94, 127-137.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4, 67-69.

Seinhorst, J.W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178.

Tiquia, S.M., Wan, J.H.C., Tam, N.F.Y., 2002. Microbial population dynamics and enzyme activities during composting. Compost Sci. Util. 10, 150-161.

Tiquia, S.M., 2005. Microbial community dynamics in manure composts based on 16S and 18S rDNA T-RFLP profiles. Environ. Technol. 26, 1101-1113.

Vallad, G.E., Cooperband, L., Goodman, R.M., 2003. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. Physiol. Mol. Plant Pathol. 63, 65-77.

Wang, K.H., McSorley, R., Marshall, A.J., Gallaher, R.N., 2004. Nematode community changes associated with decomposition of *Crotalaria juncea* amendment in litterbags. Appl. Soil Ecol. 27, 31-45.

Wasilewska, L., 1998. Changes in the proportions of groups of bacterivorous soil nematodes with different life strategies in relation to environmental conditions. Appl. Soil Ecol. 9, 215-220.

Wharton, D.A., 1986. A Functional Biology of Nematodes. Croom Helm, London and Sydney.

Yeates, G.W., 1969. Predation by *Mononchoides potohikus* (Nematoda: Diplogasteridae) in laboratory culture. Nematologica 15, 1-9.

Yeates, G.W., 1970. Studies on laboratory cultures of dune sand nematodes. J. Nat. Hist 4, 119-136.

Yeates, G.W., 1984. Variation in soil nematode diversity under pasture with soil and year. Soil Biol. Biochem. 16, 95-102.

Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biol. Fertil. Soils 37, 199-210.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera—an outline for soil ecologists. J. Nematol. 25, 315-331.

Yeates, G.W., Bongers, T., 1999. Nematode diversity in agroecosystems. Agric. Ecosyst. Environ. 74, 113-135.

Yeates, G.W., Ferris, H., Moens, T., Van der Putten, W.H., 2009. The role of nematodes in ecosystems. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, pp. 1-45.

Zucconi, F., de Bertoldi, M., 1987. Specifications for solid waste compost. Biocycle 28, 56-61.

Zvomuya, F., Larney, F.J., DeMaere, P.R., Olson, A.F., 2008. Organic amendment effects on crop productivity and nutrient uptake on reclaimed natural gas wellsites. Nutr. Cycl. Agroecosys. 80, 223-232.

3. The nematode fauna in compost

Modified from:

- Steel H, Moens T, Scholaert A, Boshoff M, Houthoofd W and Bert W (2011). *Mononchoides composticola* n. sp. (Nematoda: Diplogasteridae) associated with composting processes: morphological, molecular and autoecological characterization. Nematology 13(3): 347-363.
- Steel H, Coomans A, Moens T and Bert W. Non-marine nematodes in Belgium: an updated list with special emphasis on compost nematodes. Submitted to Zootaxa.

PART 1: NON-MARINE NEMATODES IN BELGIUM: AN UPDATED LIST WITH SPECIAL EMPHASIS ON COMPOST NEMATODES.

3.1 Abstract

A study of the semi-artificial and controlled composting process in Eastern Flanders revealed 35 different taxa of which 21 were new records Belgium. An updated checklist of terrestrial, fresh- and brackish water nematodes in Belgium is presented. The Belgian non-marine nematofauna comprises 414 taxa, representing 4 subclasses, 14 orders, and 76 families. In total 124 new taxa were added: i.e. 21 from the newly explored compost habitat, 7 from freshwater samples and 96 from published data in literature.

3.2. Introduction

The Belgian nematofauna has been relatively well studied. Coomans (1989) reviewed the nematofauna from Belgium, with exclusion of the animal parasitic nematodes. However, this list was published in the proceedings of the symposium “Invertebrates from Belgium” which is, to modern standards, an inaccessible national publication. More recently, Bert et al. (2003) published an updated list of the Tylenchomorpha from Belgium, with the addition of 42 species, based on new data together with data from Bert and Geraert (2000) and Coosemans (2002). However, for the free-living Belgian nematofauna the list of Coomans (1989) was never updated or revised to reflect recent taxonomical changes. Next to the newly explored compost habitat, as presented in this PhD, and new data from freshwater samples, a literature review on both free-living (terrestrial, freshwater, brackish water and brackish soil) and plant-parasitic nematodes has given a better insight into the diversity of the Belgian nematofauna. This resulted in a list of 414 taxa, 124 taxa of which are new compared to the list of Coomans (1989) combined with Bert et al. (2003). 21 records from compost are new to the Belgian fauna.

3.3. Materials & Methods

The species list is mainly compiled based on compost samples, on a limited number of freshwater samples and on literature data. For compost, nine composting processes were sampled at different time points, these include five processes according to the Controlled Microbial Composting method (=farm composting), three small scale processes in barrels and one industrial green waste composting process. Seven additional samples of mature composts were analyzed from a green waste process and six farm composting processes executed at the experimental farm of the Institute for Agricultural and Fisheries Research (ILVO, Merelbeke,

Belgium). For more detailed information on the used method of sampling and processing of the samples see Chapters 2, 4 & 7. The included new records of freshwater nematodes are the result of about thirty randomly taken samples from freshwater habitats (ponds, lakes, cannals, etc.).

The presented list includes the main characteristic of the site where the taxa were first recorded in Belgium, T= terrestrial, C= compost, Fw= freshwater, Bw= brackish water and Bs= brackish soil. This habitat indication is not exhaustive, presence in other habitats in- or outside Belgium is not provided. However, although this list only contains non-marine nematodes, taxa known to have also a marine distribution in- or outside Belgium are indicated with (M). Generally, the classification of De Ley and Blaxter (2004) is followed up to family level, unless stated otherwise. For the orders Triplonchida and Plectida, classifications of Holovachov and Shoshin (in press) and Holovachov (in press), respectively, were used. For the Dorylaimida the classifications of Peña-Santiago (2006) and Vinciguerra (2006) were appplied while for the Mononchida the classification of Zullini and Peneva (2006) was applied. For classification of the infraorder Tylenchomorpha Decraemer and Hunt (2006) was used. On subfamily level more specialized classifications were followed for the following taxa: Rhabditidae (Sudhaus 2011); Diplogasteridae *s.l.* (Sudhaus and Fürst von Lieven 2003); Criconematidae (Geraert 2010) and Dolichodoridae and Psilenchidae (Hunt, Bert & Siddiqi, in press).

3.4 Results & Discussion

Compost nematodes

The study of the hitherto completely unexplored compost biotope resulted in 35 different taxa 21 of which were new records for Belgium (7 species, 13 genera and 1 family) (Table 1): Neotylenchidae unidentified sp., *Nygolaimoides* sp.; *Ektaphelenchoides* sp.; *Ditylenchus filimus*; 8 rhabditids: *Crustorhabditis* sp. *Cruznema* sp., *Diploscapter coronatus*, *Parasitorhabditis* sp., *Pelodera cylindrica*, *Pelodera terres*, *Rhabditophanes cobbi*, *Teratorhabditis* sp.; 6 diplogasterids: *Acrostichus* sp., *Diplogaster* sp., *Diplogasteritus* sp., *Diplogasterioides* sp., *Fictor* sp., *Mononchoides composticola* and 3 panagrolaimids: *Panagrellus* sp., *Panagrolaimus labiatus*, *Procephalobus* sp. Other genera recorded from compost but not new for Belgium were: *Cephalobus*, *Eucephalobus*, *Aphelenchoides*, *Seinura*, *Filenchus*, *Butlerius*, *Bunonema*, *Cephaloboides*, *Choriorhabditis*, *Mesorhabditis*, *Poikilolaimus* and *Protorhabditis*. Also *Rhabditella axei* and *Halicephalobus* cfr.[4] *gingivalis* were found in compost however their recording was not new for Belgium. By far the most widespread taxa in the examined compost samples (based on presence or absence) were *Pelodera terres*, *Pelodera cylindrica*,

[4] Unpublished results indicated considerable molecular differences between species that are morphologically identified as *Halicephalobus gingivalis*.

Aphelenchoides sp*., Halicephalobus* cfr. *gingivalis*, *Poikilolaimus* sp., *Diploscapter coronatus*, *Acrostichus* sp., *Fictor* sp. and *Mononchoides composticola*. For a detailed description of the latter see Part 2 of this chapter. Remarkably, the same genera and even the same species were recorded from the very limited compost samples studied by other authors. The majority of species found were also rhabditids, panagrolaims and diplogasterids, and with even the same genera or species. *Eucephalobus*, *Diploscapter*, *Poikilolaimus* sp., *Aphelenchoides* and *Ditylenchus* were found from compost in Russia (Gagarin 2000). Identical observations on species level include *Panagrolaimus labiatus* (China, Andrássy 1984), *Ditylenchus filimus* (West-Canada, Anderson 1983) and *Halicephalobus gingivalis* (USA, Riverside, Nadler et al. 2003). The presence of a highly similar compost nematode diversity is at first sight difficult to explain given the geographic disparity and different feedstock materials. However, a compost process is a temporally vastly changing environment with food in abundance, selective for opportunistic nematodes that are highly efficient dispersers (Chapter 5). It is well-known that certain nematodes are excellent survivors (Wharton 2004) and very efficient dispersers (Hodda et al. 2009); these characteristics, among others, may cause nearly cosmopolitan distributions (Artois et al. 2011). Mark that the occurence of nematodes marked in the list as comopost nematodes is not restricted to compost. For example the cosmopolitan and free-living *Diploscapter coronatus* was described, next to a terrestrial habitat, as a facultative parasite of humans (e.g. Athari and Mahmoudi 2008) and from necrotic nodules in the skin of snakes (Sabu et al. 2002). Also *Halicephalobus gingivalis* is a free-living terrestrial panagrolaimid that is also capable of infecting and reproducing in horses, humans and zebras (Nadler et al. 2003). Other taxa from compost have a more specific biology, such as Neotylenchidae sp., which has alternating life cycles (parasitic in the insect haemocoel and a free-living, fungal- or plant-feeding generation) or *Ektaphelenchoides* sp., which is known from insects associations in xylem of several woods (Siddiqi 2000 and Hunt 1993 respectively).

Literature review and freshwater study

The review of Coomans (1989) and Bert et al. (2003) together listed 290 taxa and another 124 have now been added (Table 3.1). Seven nominal species have been removed from the 1989 list of Coomans because they have been synonymized with other species on the list (i.e. *Cervidellus serratus, Basiria minor, Filenchus filiformis, Meloidogyne deconincki, Neopsilenchus minor, Rotylenchus fallorobustus* and *Tylenchorinchus judithae*).

Next to the Tylenchomorpha already added in the list of Bert et al. (2003) the study of Coosemans (2002) reported 16 other new records for Belgium. A study on historical pollution (Bert et al. 2009) and one on the effect of exogenous organic matter on the nematode

community in agricultural soil (Leroy et al. 2009) revealed 4 and 6 new genera or taxa respectively. The works of Andrássy 2005 and 2007 together mentioned 2 free-living nematode species (i.e. *Monhystera paludicola* and *Cylindrolaimus melancholicus*) and 3 plant-parasitic species (*Scutylenchus quadrifer*, *Scutylenchus tessellates* and *Criconemoides annulatus*) that had not previously been recorded and according to Tiasi *et al.* (2010), *Mylonchulus sigmaturellus* was also found in Belgium. Although *Nanidorus minor* is mentioned from Belgium (Andrássy 2009), this species is not added to the list because its identification is probably not correct and should be *N. renifer* (Braasch and Sturhan 1991). Concerning the plant-parasitic nematodes, Bongers (1994) and an additional 12 studies (Elbadri et al. 1999; Subbotin et al. 2000; Karssen et al. 2000; Rubtsova et al. 2001; Subbotin et al. 2003; Wobalem 2004; Madan et al. 2005; Kovaleva et al. 2005; Viaene et al. 2007; Leroy et al. 2009; Sturhan and Hallmann 2010; Vandenbossche et al. 2011) together yielded 28 new records for Belgium, which now have been added to the list. Six new records of entomopathogenic species were provided by Miduturi et al. (1996), Spiridonov and Moens (1999), Ansari *et al.* (2003) and Ansari *et al.* (2007): *Steinernema affine, S. carpocapsae, S. feltiae, S. kraussei* and *Heterorhabditis bacteriophora* and *H. megidis*. A study based on two Belgian brackish water stations in the river Scheldt by De Block (1994) resulted in 23 additional new records for Belgium.

The freshwater nematode species list was updated with 5 new species from farmland ponds (Bert et al. 2007). Additional sampling of several ponds, lakes, and canals in Belgium resulted in 7 new records. *Hirschmanniella behningi* was found from a very small pond, in De Haan (51°16'57.01"N; 3° 3'55.33"E; voucher UGnem79), the pond has been muted in the meantime. *Eutobrilus nothus* and *Semitobrilus pellucidus* were recorded from a small lake in Geel (51°10'23.96"N; 5° 2'34.70"E). *Limnomermis* sp. was found in the river “kleine Nete” in Grobbendonk (51°11'5.71"N; 4°47'1.78"E). *Rhabdolaimus terrestris* and *Chromadorina bioculata* were recovered from lakes that originate from the excavation of white sand in Mol (51°13'48.95"N; 5°10'37.14"E) and Dessel (51°15'47.89"N; 5° 8'15.60"E) respectively. *Panagrolaimus thienemanni* (synonym of *Propanagrolaimus filiformis*) was found in an artificial fish pond in Geel (51°7'37.25"N; 4°59'22.59"E). A series of digital images and the SSU rDNA of each of the above freshwater species is available (Helder et al., unpublished).

3.5. Conclusion

In total 124 new records were added to the list of Coomans (1989) combined with Bert et al. (2003): i.e. 21 from the newly explored compost habitat, 7 from freshwater samples and 96 from published data in literature. Although the diversity in the compost samples itself was relatively low (only 2-10 different taxa per sample), more than half of the recorded taxa were new (21 out

of 35) for Belgium. This indicates that the free-living terrestrial taxa, especially from poorly investigated habitats, are still underrepresented and that investigation of other unexplored habitats will easily reveal more new records for Belgium.

1

Table 3.1:
Updated list of the nematode taxa from Belgium. The main characteristic of the site where the taxa were first recorded in Belgium is indicated by T= terrestrial, C= compost, Fw= fresh water, Bw= Brackish water, Bs= Brackish soil or U= unknown. Taxa known to also have a marine distribution in-or outside Belgium are indicated with (M). Numbers refer to studies were the taxa are mentioned; 1: Coomans (1989); 2: Geraert et al. (1988); 3: Bongers (1999); 4: De Block (1994); 5: Miduturi et al. (1996); 6: Spiridonov and Moens (1999); 7: Elbadri et al. (1999); 8: Bert and Geraert (2000); 9: Subbotin et al. (2000); 10: Karssen et al. (2000); 11: Rubtsova et al. (2001); 12: Coosemans (2002); 13: Bert et al. (2003); 14: Subbotin et al. (2003); 15: Ansari et al. (2003); 16: Wobalem (2004); 17: Andrássy (2005); 18: Madan et al. (2005); 19: Kovaleva et al. (2005); 20: Ansari et al. (2007); 21: Bert et al. (2007); 22: Andrássy (2007); 23: Viaene et al. (2007); 24: Bert et al. (2009); 25: Leroy et al. (2009); 26: Tiasi et al. (2010); 27: Sturhan and Hallmann (2010); 28: Vandenbossche et al. (2011); 29: Chapter 3 part 2; 30: new data from freshwater samples; 31: present data on compost nematofauna; 32: Fonderie et al., in press.

Class Enoplea	**Site**	**Ref.**
Subclass Enoplia		
Order Enoplida		
Suborder Enoplina		
Family Anoplostomatidae		
Anoplostoma campbelli Allgén, 1932	Bs Bw (M)	1
Anoplostoma viviparum (Bastian, 1865) Bütschli, 1874	Bw (M)	4
Suborder Oncholaimina		
Family Oncholaimidae		
Adoncholaimus thalassophygas (de Man, 1876)	Bw Bs (M)	1
Oncholaimus oxyuris Ditlevsen, 1911	Bw Bs (M)	1
Viscosia viscosa (Bastian, 1865) de Man, 1890	Bw (M)	4
Family Enchelidiidae		
Calyptronema	Bw (M)	4
Suborder Ironina		
Family Ironidae		
Syringolaimus striatocaudatus de Man, 1888	Bs (M)	1
Family Oxystominidae		
Halalaimus gracilis de Man, 1888	Bw (M)	4
Nemanema	Bw (M)	4
Suborder Tripyloidina		
Family Tripyloididae		
Tripyloides gracilis (Ditlevsen, 1918)	Bw (M)	4
Suborder Alaimina		
Family Alaimidae		
Alaimus mucronatus Altherr, 1950	T	12
Alaimus proximus Thorne, 1939	T	12
Alaimus primitivus de Man, 1880	T	1
Amphidelus elegans (de Man, 1921) Thorne, 1939	T	1
Paramphidelus dolichurus (de Man, 1876) Andrássy, 1977	T	1
Paramphidelus uniformis (Thorne, 1939) Andrássy, 1977	T	1
Order Triplonchida		
Suborder Diptherophoroidea		

Family Diptherophoridae		
Diphtherophora communis de Man, 1880	T	1
Diphtherophora vanoyei De Coninck, 1931	T Fw	1
Tylolaimophorus typicus de Man, 1880	T	1
Family Trichodoridae		
Nanidorus nanus (Allen, 1957) Siddiqi, 1974	T	1
Paratrichodorus pachydermus (Seinhorst, 1954) Siddiqi, 1974	T	1
Paratrichodorus teres (Hooper, 1972) Siddiqi, 1974	T	1
Trichodorus cylindricus Hooper, 1962	T	1
Trichodorus primitivus (de Man, 1880) Micoletzky, 1922	T	1
Trichodorus similis Seinhorst, 1963	T	1
Trichodorus sparsus Szczygiel, 1968	T	1
Trichodorus variopapillatus Hooper, 1972	T	1
Trichodorus velatus Hooper, 1972	T	1
Trichodorus viruliferus Hooper, 1963	T	1
Suborder Tobrilina		
Family Bastianiidae		
Bastiania gracilis de Man, 1876	T	1
Family Prismatolaimidae		
Prismatolaimus dolichurus de Man, 1880	Fw T	1
Prismatolaimus intermedius (Bütschli, 1873) de Man, 1880	T	1
Family Tobrilidae		
Brevitobrilus stefanskii (Micoletzky, 1925) Tsalolikhin, 2002	Fw	21
Eutobrilus nothus Gagarin, 1989	Fw	30
Neotobrilus diversipapillatus (Daday, 1905) Tsalolikhin, 1981	Fw	21
Semitobrilus pellucidus (Bastian, 1865) Tsalolikhin, 1981	Fw	30
Tobrilus gracilis (Bastian, 1865) Andrássy, 1959	Fw T	1
Suborder Tripylina		
Family Tripylidae		
Tripyla filicaudata de Man, 1880	T	1
Tripyla glomerans Bastian, 1865	Fw T Bw	1
Tripyla setifera Bütschli, 1873	T	1
Subclass Dorylaimia		
Order Dorylaimida		
Suborder Dorylaimina		
Family Belondiridae		
Axonchium coronatum (de Man, 1906) Thorne & Swanger, 1936	T	3
Dorylaimellus demani Goodey, 1963	T	1
Oxydirus Thorne, 1939	T	25
Family Mydonomidae		
Dorylaimoides limnophilus (de Man, 1880) Loof, 1964	T	1
Family Tylencholaimellidae		
Tylencholaimellus striatus Thorne, 1939	T	1
Family Tylencholaimidae		
Tylencholaimus mirabilis (Bütschli, 1873) de Man, 1876	T	1
Tylencholaimus proximus Thorne, 1939	T	1

Tylencholaimus stecki Steiner, 1914	T	1
Suborder Nygolaimina		
Family Actinolaimidae		
Paractinolaimus macrolaimus (de Man, 1880) Andrássy, 1964	Fw T	1
Family Aporcelaimidae		
Aporcelaimellus obscurus (Thorne & Swanger, 1936) Heyns, 1965	T	1
Aporcelaimellus obtusicaudatus (Bastian, 1865) Heyns, 1965	T Fw Bw	1
Aporcelaimellus tritici (Bastian, 1865) Andrássy, 1986	T Fw	1
Aporcelaimus regius (de Man, 1880) Thorne & Swanger, 1936	T	1
Family Dorylaimidae		
Chrysodorus filiformis (Bastian, 1865) Andrássy, 1988	T	1
Dorylaimus crassus de Man, 1884	T	1
Dorylaimus stagnalis Dujardin, 1845	Fw	21
Mesodorylaimus bastiani (Bütschli, 1873) Andrássy, 1959	T Bw	1
Prodorylaimium brigdammense (de Man, 1876) Andrássy, 1969	T Fw	1
Prodorylaimus rotundiceps Loof, 1985	T	12
Family Longidoridae		
Longidorus attenuates Hooper, 1961	T	1
Longidorus caespiticola Hooper 1961	T	1
Longidorus cylindricaudatus Korlowska & Seinhorst, 1979	T	1
Longidorus elongatus (de Man, 1876) Thorne & Swanger, 1936	T	1
Longidorus goodeyi Hooper, 1961	T	1
Longidorus intermedius Korlowska & Seinhorst, 1979	T	1
Longidorus leptocephalus Hooper, 1961	T	1
Longidorus macrosoma Hooper, 1961	T	1
Longidorus profundorum Hooper, 1966	T	1
Longidorus sturhani Robustova, Subbotin, Brown & Moens, 2001	T	11
Longidorus vineacola Sturhan & Weischer, 1964	T	1
Paralongidorus maximus (Buetschli, 1874) Siddiqi, 1964	T	3
Xiphinema coxi Tarjan, 1964	T	1
Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939	T	1
Family Nordiidae		
Dorydorella pratensis (de Man, 1880) Andrássy, 1986	T Bs	1
Longidorella parva Thorne, 1939	T	1
Pungentus engadinensis (Altherr, 1950) Altherr, 1952	T	1
Pungentus silvestris (de Man, 1912) Coomans & Geraert, 1962	T	1
Family Qudsianematidae		
Crassolabium	T	24
Ecumenicus monohystera (de Man, 1880) Thorne, 1974	T	1
Epidorylaimus consobrinus (de Man, 1918) Andrássy, 1986	T	1
Eudorylaimus acuticauda (de Man, 1880) Andrássy, 1986	T Bs	1
Eudorylaimus carteri (Bastian, 1865) Andrássy, 1959	T Fw Bs Bw	1
Eudorylaimus centrocercus (de Man, 1880) Andrássy, 1959	T	1
Labronema vulvapapillatum (Meyl, 1954) Loof & Grootaert, 1981	T	3
Microdorylaimus modestus (Altherr, 1952) Jairajpuri, 1970	T	12
Family Thornenematidae		

Ophistodorylaimus sylphoides (Williams, 1959) Carbonell & Coomans, 1986	T	12
Family Thorniidae		
Nygolaimoides	**C**	**31**
Thornia	T	24
Family Nygolaimidae		
Nygolaimus	T	24
Order Mononchida		
Suborder Mononchina		
Family Anatonchidae		
Anatonchus tridentatus (de Man, 1876) Cobb, 1916	T	1
Family Mononchidae		
Clarkus papillatus (Bastian, 1865) Jairajpuri, 1970	T	12
Coomansus parvus (de Man, 1880) Jairajpuri & Khan, 1977	T	25
Mononchus aquaticus Coetzee, 1968	Fw	1
Mononchus tunbridgensis Bastian, 1865	Fw	21
Prionchulus muscorum (Dujardin, 1845) Cobb, 1916	T	1
Prionchulus punctatus Cobb, 1917	T	1
Family Mylonchulidae		
Mylonchulus brachyuris (Bütschli, 1873) Cobb, 1917	T	1
Mylonchulus sigmaturellus Mulvey, 1961	T	26
Order Isolaimida		
Family Isolaimiidae		
Isolaimium multistriatum Hogewind & Heyns, 1967	T	1
Order Mermithida		
Family Mermithidae		
Limnomermis	Fw	30
Class Chromadorea		
Subclass Chromadoria		
Order Desmoscolecida		
Suborder Desmoscolecida		
Family Desmoscolecidae		
Desmoscolex vinealis Weischer, 1962	T (M)	3
Order Plectida		
Family Aphanolaimidae		
Aphanolaimus aquaticus Daday, 1894	T Fw	1
Aphanolaimus attentus de Man, 1880	T Fw	1
Aphanolaimus deconincki Coomans & De Waele, 1983	T Fw	1
Aphanolaimus pseudoattentus Coomans & De Waele, 1983	T	1
Family Camacolaimidae		
Deontolaimus papillatus de Man, 1880	Bs (M)	1
Camacolaimus tardus de Man, 1889	Bw (M)	4
Family Chronogasteridae		
Chronogaster typica de Man, 1921	Fw	1
Family Leptolaimidae		
Antomicron elegans (de Man, 1922)	Bw (M)	4

Leptolaimus papilliger de Man, 1876	Bw (M)	4
Family Metateratocephalidae		
Metateratocephalus crassidens (de Man, 1880) Eroshenko, 1973	T	1
Family Plectidae		
Anaplectus granulosus (Bastian, 1865) De Coninck & Schuurmans Stekhoven, 1933	T Bs	1
Plectus acuminatus Bastian, 1865	T	12
Plectus armatus (Bütschli, 1873) Andrássy, 1984	T Bw	1
Plectus cirratus Bastian, 1865	Fw T Bw	1
Plectus geophilus de Man, 1880	T	1
Plectus parvus Bastian, 1865	T	1
Plectus longicaudatus Bütschli, 1873	T	1
Plectus parietinus Bastian, 1865	T	12
Plectus pusillus Cobb, 1893	T	1
Plectus rhizophilus de Man, 1880	T	1
Plectus tenuis Bastian, 1865	T	25
Tylocephalus auriculatus (Bütschli, 1873) Crossman, 1933	T	1
Wilsonema otophorum (de Man, 1880) Cobb, 1913	T	1
Wilsonema schuurmansstekhoveni (De Coninck, 1931) Zell, 1982	T	3
Family Rhabdolaimidae		
Rhabdolaimus terrestris de Man, 1880	Fw	30
Order Araeolaimida		
Family Axonolaimidae		
Axonolaimus paraspinosus Schuurmans Steckhoven & Adam 1931	Bs (M)	4
Family Comesomatidae		
Sabatieria pulchra (Schneider, 1906)	Bs (M)	4
Family Diplopeltidae		
Cylindrolaimus communis de Man, 1880	T	1
Cylindrolaimus melancholicus de Man, 1880	T	17
Southerniella	Bs (M)	4
Order Monhysterida		
Suborder Monhysterina		
Family Monhysteridae		
Diplolaimella allgeni Schneider, 1967	Bw	4
Diplolaimella dievengatensis Jacobs, Van de Velde, Geraert & Vranken, 1990	Bw Bs	1
Diplolaimella ocellata (Bütschli, 1874) Gerlach, 1957	Bw	1
Eumonhystera filiformis (Bastian, 1865) Andrássy, 1981	Fw Bs Bw T	1
Eumonhystera vulgaris (de Man, 1880) Andrássy, 1981	T	1
Geomonhystera villosa (Bütschli, 1873) Andrássy, 1981	T	1
Halomonhystera disjuncta (Bastian, 1865) Andrássy, 2006	Bs Bw (M)	1
Monhystera microphthalma de Man, 1880	Bw (M)	1
Monhystera paludicola de Man, 1981	T	15
Monhystera riemanni Jacobs & Heyns, 1987	Fw	21
Monhystrella macrura (de Man, 1880) Andrássy, 1981	Bs Bw	1
Monhystrella parelegantula (De coninck, 1943) Andrássy, 1981	Bw Bs	1

Thalassomonhystera parva (Bastian, 1865) Jacobs, 1987	Bw Bs (M)	1
Family Spaerolaimidae		
Sphaerolaimus gracilis de Man, 1876	Bs (M)	1
Family Xyalidae		
Daptonema setosum (Bütschli, 1874)	Bw (M)	4
Theristus longisetosus (Schuurmans Stekhoven & De Coninck, 1933)	Bw (M)	1
Theristus velox (Bastian, 1865)	Bs Bw	1
Suborder Linhomoeina		
Family Linhomoeidae		
Desmolaimus zeelandicus de Man 1880	Bw (M)	4
Order Desmodorida		
Suborder Desmodorina		
Family Microlaimidae		
Calomicrolaimus honestus (de Man, 1922)	Bw (M)	4
Microlaimus acuticaudatus Schuurmans Stekhoven & De Coninck, 1933	Bw (M)	1
Microlaimus globiceps de Man, 1880	Bw Bs (M)	1
Microlaimus robustidens Schuurmans Stekhoven & De Coninck, 1933	Bw (M)	1
Prodesmodora	T (M)	24
Order Chromadorida		
Suborder Chromadorina		
Family Achromadoridae		
Achromadora ruricola (de Man, 1880) Micoletzky, 1925	T	1
Family Chromadoridae		
Chromadorina bioculata (Schultze In Carus, 1857) Wieser, 1954	Fw (M)	30
Dichromadora geophila (de Man, 1876) Kreis, 1929	Bw (M)	4
Hypodontolaimus balticus (Schneider, 1906) Filipjev, 1918	Bw (M)	4
Neochormadora complexa Gerlach, 1953	Bw (M)	4
Ptycholaimellus ponticus (Filipjev, 1922) Gerlach, 1955	Bw (M)	4
Spilophorella paradoxa (de Man, 1888) Filipjev, 1917	Bw (M)	4
Family Cyatholaimidae		
Paracanthonchus caecus (Bastian, 1865) Micoletzky, 1924	Bw (M)	4
Paracyatholaimus intermedius (de Man 1880) Filipjev, 1930	Bw T (M)	1
Family Ethmolaimidae		
Ethomolaimus	Bw (M)	4
Order Rhabditida		
Incertae sedis: Family Teratocephalidae		
Teratocephalus terrestris (Bütschli, 1873) de Man, 1876	T	1
Teratocephalus costatus Andrássy, 1958	T	12
Suborder Tylenchina		
Infraorder Panagrolaimorpha		
Family Panagrolaimidae		
***Halicephalobus* cfr. *gingivalis* Stefanski, 1954**	**T C**	**32**
Halicephalobus laticauda Geraert, Sudhaus, Lenaerts & Bosmans, 1988	T	2
Panagrolaimus rigidus (Schneider, 1866) Thorne, 1937	T Fw Bw Bs	1
***Panagrolaimus labiatus* (Kreis, 1929) Andrássy, 1960**	**C**	**31**
Panagrolaimus subelongatus (Cobb, 1914) Thorne, 1937	T	12

Propanagrolaimus filiformis (de Man, 1880) Andrássy, 2005	Fw	30
Procephalobus	**C**	**31**
Panagrellus	**C**	**31**
Family Steinernematidae		
Heterorhabditis bacteriophora Poinar, 1976	T	15
Heterorhabditis megidis Poinar, Jackson & Klein, 1987	T	15
Steinernema affine (Bovien, 1937) Wouts, Mrácek, Gerdin & Bedding, 1982	T	5
Steinernema carpocapsae Weiser, 1955	T	20
Steinernema feltiae (Filipjev, 1934) Wouts, Mrácek, Gerdin & Bedding, 1982	T	5
Steinernema kraussei (Steiner, 1923) Travassos, 1927	T	6
Infraorder Cephalobomorpha		
Family Cephalobidae		
Acrobeles ciliatus v. Linstov, 1877	T	1
Acrobeloides nanus (de Man, 1880) Thorne, 1937	T	1
Acrolobus emarginatus (de Man, 1880) Thorne, 1937	T	1
Acrobelophis minimus (Thorne, 1937) Andrássy, 1984	T	1
Acrobelophis deconincki (Coomans, 1962) Vinciguerra & Clausi, 1996	T	1
Cephalobus persegnis Bastian, 1865	T	1
Cervidellus vexilliger (de Man, 1880) Thorne, 1937	T	1
Chiloplacus demani (Thorne, 1925) Schneider, 1939	T	1
Eucephalobus oxyuroides (de Man, 1876) Steiner 1936	T Bw	1
Eucephalobus striatus (Bastian, 1865) Thorne, 1937	T Fw Bw Bs	1
Heterocephalobus elongatus (de Man, 1880) Andrássy, 1967	T Bw	1
Heterocephalobus longicaudatus (Bütschli, 1873)	T	25
Seleborca complexus (Thorne, 1925) Andràssy, 1985	T	1
Seleborca mariannae (Andrássy, 1968) Andrássy, 1985	T	12
Family Osstellidae		
Drilocephalobus goodeyi Suryawanshi & Christy, 1973	T	1
Infraorder Tylenchomorpha		
Family Anguinidae		
Ditylenchus destructor Thorne, 1945	T	13
Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936	T	1
***Ditylenchus filimus* Anderson, 1983**	**C**	**31**
Ditylenchus intermedius (de Man, 1880) Filipjev, 1936	T	1
Pseudhalenchus minutus Tarjan, 1958	T	1
Subanguina radicicola (Greeff, 1872) Paramonov, 1968	T	1
Family Aphelenchidae		
Aphelenchus avenae Bastian, 1865	T	1
Family Aphelenchoididae		
Aphelenchoides asterocaudatus Das, l960	T	13
Aphelenchoides bicaudatus (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941	T	1
Aphelenchoides blastopthorus Franklin, 1952	T	13
Aphelenchoides composticola Franklin, 1957	T	1
Aphelenchoides fragariae (Ritzema Bos, 1890) Christie, 1932	T	1
Aphelenchoides parietinus (Bastian, 1865) Steiner, 1932	T	1

Aphelenchoides ritzemabosi (Schwartz, 1911) Steiner & Buhrer, 1932	T	1
Aphelenchoides subtenuis (Cobb, 1926) Steiner & Buhrer, 1932	T	1
Aprutides guidettii Scognamiglio, 1974	T	12
Ektaphelenchoides	**C**	**31**
Laimaphelenchus penardi (Steiner, 1914) Filipjev & Schuurmans Stekhoven, 1941	T	13
Seinura diversa (Paesler, 1957) Goodey, 1960	T	1
Family Criconematidae		
Criconema annuliferum (de Man, 1921) Micoletzky, 1925	T	1
Criconema demani Micoletzky, 1925	T	8
Criconema longulum Gunhold, 1953	T	1
Criconema loofi (De Grisse, 1967) Raski & Luc, 1985	T	1
Criconema mutabile (Taylor, 1936) Raski & Luc, 1985	T	12
Criconema princeps (Andràssy, 1962) Raski & Luc, 1985	T	1
Criconema sphagni Micoletzky, 1925	T	1
Criconemoides amorphus De Grisse, 1967	T	1
Criconemoides annulatus Cobb in Taylor, 1936	T	22
Criconemoides informis (Micoletzky, 1922) Taylor, 1936	T	1
Criconemoides morgensis (Hofmänner *in* Hofmänner & Menzel, 1914) Taylor, 1936	T	1
Criconemoides parvus Raski, 1952	T	1
Crossonema menzeli (Stefanski, 1924) Metha & Raski, 1971	T	1
Hemicriconemoides pseudobrachyurus De Grisse, 1964	T	1
Mesocriconema axeste (Fassuliotis & Williamson, 1959) Loof & De Grisse, 1989	T	1
Mesocriconema crenatum (Loof, 1964) Andrássy, 1965	T	1
Mesocriconema curvatum (Raski, 1952) Loof & De Grisse, 1989	T	1
Mesocriconema dherdei (De Grisse, 1967) Loof & De Grisse, 1989	T	1
Mesocriconema irregulare (De Grisse, 1964) Loof & De Grisse, 1989	T	1
Mesocriconema kirjanovae (Andrássy, 1962) Loof & De Grisse, 1989	T	8
Mesocriconema maritimum (De Grisse, 1964) Loof & De Grisse, 1989	T	1
Mesocriconema ornatum (Raski, 1952) Loof & De Grisse, 1989	T	1
Mesocriconema pseudosolivagum (De Grisse, 1964) Loof & De Grisse, 1989	T	1
Mesocriconema raskiense(De Grisse, 1964) Andrássy, 1965	T	1
Mesocriconema rotundicauda (Loof, 1964) Loof, 1989	T	1
Mesocriconema rusticum (Micoletzky, 1915) Loof & De Grisse, 1989	T	1
Mesocriconema solivagum (Andrássy, 1962) Loof & De Grisse, 1989	T	1
Mesocriconema sphaerocephala (Taylor, 1936) Loof, 1989	T	1
Mesocriconema vadense (Loof, 1964) Loof & De Grisse, 1989	T	1
Mesocriconema xenoplax (Raski, 1952) Loof, 1989	T	1
Ogma cobbi (Micoletzky, 1925) Siddiqi, 1986	T	1
Xenocriconemella macrodora (Taylor, 1936) De Grisse & Loof, 1965	T	1
Family Dolichodoridae		
Amplimerlinius icarus (Wallace & Greet, 1964) Siddiqi, 1976	T	8
Amplimerlinius macrurus (Goodey, 1932) Siddiqi, 1976	T	1

Macrotrophurus arbusticola Loof, 1958	T	1
Merlinius brevidens (Allen, 1955) Brzeski, 1991	T	1
Merlinius joctus (Thorne, 1949) Brzeski, 1991	T	1
Merlinius microdorus (Geraert, 1966) Brzeski, 1991	T	1
Merlinius nanus (Allen, 1955) Brzeski, 1991	T	1
Merlinius nothus (Allen, 1955) Brzeski, 1991	T	1
Nagelus alpensis Doucet & Luc, 1981	Fw	3
Nagelus obscurus (Allen, 1955) Powers, Baldwin & Bell, 1983	Fw	8
Neodolichodorus lamelliferus (de Man, 1880) Filipjev, 1936	T	1
Neodolichodorus microphasmis Loof, 1960	T	1
Quinisulcius capitatus Allen, 1955	T	1
Scutylenchus quadrifer (Andràssy, 1954) Brzeski, 1991	T	1
Scutylenchus tessellatus (Goodey, 1952) Brzeski, 1991	T	1
Telotylenchus ventralis (Loof, 1963) Fortuner & Luc 1987	T	1
Tylenchorhynchus claytoni Steiner, 1937	T	1
Tylenchorhynchus contractus Loof, 1964	T	3
Tylenchorhynchus dubius (Bütschli, 1873) Filipjev, 1936	T	1
Tylenchorhynchus maximus Allen, 1955	T	1
Family Hemicycliophoridae		
Hemicycliophora conida Thorne, 1955	T	1
Hemicycliophora similis Thorne, 1955	T	1
Hemicycliophora thienemanni (Schneider,1952) Loof, 1984	T	28
Hemicycliophora triangulum Loof, 1968	T	8
Family Hoplolaimidae		
Globodera pallida (Stone, 1973) Behrens, 1975	T	18
Globodera rostochiensis (Wollenweber, 1923) Behrens, 1975	T	1
Helicotylenchus canadensis Waseem, 1961	T	13
Helicotylenchus exallus Sher, 1966	U	8
Helicotylenchus minzi Sher, 1966	T	1
Helicotylenchus multicinctus (Cobb, 1893) Golden, 1956	T	1
Helicotylenchus pseudorobustus (Steiner, 1914) Golden, 1956	T	1
Helicotylenchus varicaudatus Yuen, 1964	T	8
Heterodera aucklandica Wouts & Sturhan, 1995	T	14
Heterodera avenae Wollenweber, 1924	T	1
Heterodera bifenestra Cooper, 1955	T	28
Heterodera cruciferae Franklin, 1945	T	1
Heterodera goettingiana Liebscher, 1892	T	1
Heterodera hordecalis Andersson, 1975	T	28
Heterodera humuli Filipjev, 1934	T	1
Heterodera mani Matthews, 1971	T	28
Heterodera ripae Subbotin, Sturhan, Rumpenhorst & Moens, 2003	T	19
Heterodera salixophila Kirjanova, 1969	T	9
Heterodera schachtii Schmidt, 1871	T	1
Heterodera trifolii Goffart, 1932	T	1
Heterodera ustinovi Kirjanova, 1969	T	14

Heterodera utricae Cooper, 1955	T	9
Meloidodera alni Turkina & Chizhov, 1986	T	9
Peltamigratus parapachyuris Rashid, 1986	T	3
Punctodera punctata (Thorne, 1928) Mulvey & Stone, 1976	T	1
Rotylenchus goodeyi Loof & Oostenbrink, 1958	T	1
Rotylenchus robustus (de Man, 1880) Filipjev, 1936	T	1
Rotylenchus uniformis (Thorne, 1949) Loof & Oostenbrink, 1958	T	1
Rotylenchulus borealis Loof & Oostenbrink, 1962	T	28
Scutylenchus quadrifer (Andrássy, 1954) Siddiqi, 1979	T	22
Scutylenchus tessellates (Goodey, 1952) Siddiqi 1979	T	22
Family Meloidogynidae		
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949 [greenhouse]	T	16
Meloidogyne ardenensis Santos, 1968	T	1
Meloidogyne chitwoodi (Golden, O' Bannon, Santo and Finley, 1980) O' Bannon, Santo &Finley, 1980	T	8
Meloidogyne duytsi Karssen, Van Aelst & Van Der Putten, 1998	T	8
Meloidogyne fallax Karssen, 1996	T	8
Meloidogyne hapla Chitwood, 1949	T	1
Meloidogyne maritima (Jepson, 1987) Karssen, van Aelst & Cook, 1998	T	8
Meloidogyne minor Karssen, Bolk, van Aelst, Van den Beld, Kox, Korthals, Molendijk, Zijlstra, van Hoof & Cook 2004	T	23
Meloidogyne naasi Franklin, 1965	T	1
Family Neotylenchidae		
Unidentified	**C**	**31**
Family Pratylenchidae		
Hirschmanniella behningi (Micoletzky, 1923) Luc &Goodey, 1964	Fw	30
Hirschmanniella caudacrena Sher, 1968	Fw	27
Hirschmanniella gracilis (de Man, 1880) Luc &Goodey, 1964	T	8
Hirschmanniella loofi Sher, 1968	T	8
Hoplotylus femina s'Jacob, 1959	T	12
Pratylenchoides crenicauda Winslow, 1958	T	1
Pratylenchoides laticauda Braun & Loof, 1966	T	28
Pratylenchoides magnicauda (Thorne, 1953) Baldwin, Luc & Bell, 1983	T	13
Pratylenchus brzeskii Karssen, Waeyenberge & Moens, 2000	T	10
Pratylenchus crenatus Loof, 1960	T	1
Pratylenchus dellatrei Luc, 1958	T	1
Pratylenchus fallax Seinhorst, 1968	T	1
Pratylenchus flakkensis Seinhorst, 1968	T	8
Pratylenchus neglectus (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941	T	1
Pratylenchus penetrans (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941	T	1
Pratylenchus pratensis (De Man, 1880) Filipjev, 1936	T	1
Pratylenchus pseudopratensis Seinhorst, 1968	T	1
Pratylenchus thornei Sher & Allen, 1953	T	1
Pratylenchus vulnus Allen & Jensen, 1951	T	1

Radopholus similis (Cobb, 1893) Thorne, 1949	T	7
Family Psilenchidae		
Psilenchus aestuarius Andrássy, 1962	T	13
Psilenchus clavicaudatus (Micoletzky, 1922) Thorne, 1949	T	1
Psilenchus hilarulus de Man, 1921	T	1
Psilenchus terrextremus Hagemeyer & Allen, 1952	T	25
Family Sphaerulariidae		
Prothallonema consobrinum (de Man, 1907) Siddiqi, 1986	T	1
Family Tylenchidae		
Aglenchus agricola (de Man, 1884) Andrássy, 1954	T Fw Bw Bs	1
Basiria aberrans (Thorne, 1949) Siddiqi, 1963	T	1
Basiria duplexa (Hagemeyer & Allen, 1952) Geraert, 1968	T	1
Basiria flandriensis Geraert, 1968	T	1
Basiria gracilis (Thorne, 1949) Siddiqi, 1963	T	1
Basiria graminophila Siddiqi, 1951	T	8
Boleodorus clavicaudatus (Thorne, 1941) Mathur, Khan & Prasad, 1966	T	1
Boleodorus thylactus Thorne, 1941	T	1
Boleodorus volutus Lima & Siddigi, 1963	T	1
Cephalenchus hexalineatus (Geraert, 1962) Geraert & Goodey, 1964	T	13
Cephalenchus leptus Siddiqi, 1963	T	8
Coslenchus alacinatus Siddiqi, 1981	U	8
Coslenchus andrassyi Brzeski, 1987	T	13
Coslenchus costatus (de Man, 1921) Siddiqi, 1978	T	1
Coslenchus polonicus Brzeski, 1982	T	8
Ecphyadophora tenuissima de Man, 1921	T	1
Filenchus baloghi (Andrássy, 1958) Siddiqi, 1986	T	1
Filenchus discrepans(Andrássy, 1954) Raski & Geraert, 1987	T	12
Filenchus helenae (Szczygiel, 1969) Raski & Geraert, 1987	T	12
Filenchus misellus (Andrássy, 1958) Raski & Geraert, 1987	T	12
Filenchus quartus Szczygiel, 1969	T	1
Filenchus sandneri (Wasilewska, 1965) Raski & Geraert, 1987	T	13
Filenchus terrestris Raski & Geraert, 1987	T	12
Filenchus thornei Andràssy, 1954	T	1
Filenchus vulgaris (Brzeski, 1963) Lownsbery & Lownsbery, 1985	T	8
Irantylenchus vicinus (Szczygiel, 1970) Brzeski & Sauer, 1983	T	1
Lelenchus leptosoma (de Man, 1880) Andrássy, 1954	T	1
Malenchus acarayensis Andrássy, 1968	U	8
Malenchus andrassyi Merny, 1970	T	12
Malenchus bryophilus (Steiner, 1914) Andrássy, 1980	T	1
Miculenchus salvus Andrássy, 1959	T	1
Tylenchus arcuatus Siddiqi, 1963	T Fw	8
Tylenchus davainei Bastian, 1965	T	1
Tylenchus elegans de Man, 1876	T	13
Neopsilenchus magnidens (Thorne, 1949) Thorne & Malek, 1968	T	1
Family Tylenchulidae		

Paratylenchus aculentus Brown, 1959	T	8
Paratylenchus goodeyi Oostenbrink, 1953	T	1
Paratylenchus macrodorus Brzeski, 1963	T	1
Paratylenchus microdorus Andràssy, 1959	T	1
Paratylenchus nanus Cobb, 1923	T	1
Paratylenchus projectus Jenkins, 1956	T	1
Paratylenchus hamatus Thorne & Allen, 1950	T	1
Paratylenchus similis Khan, Prasad & Mathur, 1967	T	8
Paratylenchus straeleni (De Coninck, 1931) Oostenbrink, 1960	T	1
Suborder Rhabditina		
Infraorder Diplogasteromorpha		
Family Diplogasteridae		
Achrostichus	**C**	**31**
Butlerius degrissei (Grootaert & Jaques, 1979) Ebsary, 1986	T	1
Butlerius butleri Goodey, 1929	T	25
Diplogaster	**C**	**31**
Diplogasteritus	**C**	**31**
Fictor	**C**	**31**
Paroigolaimella	T	25
Tylopharynx foetida (Bütschli, 1874) Goodey, 1928	T	1
Family Diplogasteroididae		
Diplogasteroides	**C**	**31**
Family Neodiplogasteridae		
***Mononchoides composticola* Steel, Scholaert, Boshoff, Houthoofd & Bert, 2011**	**C**	**29**
Pristionchus lheritieri (Maupas, 1919) Paramonov, 1952	T	1
Infraorder Bunonematidomorpha		
Family Bunonematidae		
Bunonema reticulatum Richters, 1905	T	1
Bunonema richtersi Jägerskiöld, 1901	T	12
Craspedonema	T	12
Infraorder Rhabditomorpha		
Family Rhabditidae		
Cephaloboides curvicaudatus (Schneider, 1866) Dougherty, 1953	T	1
Choriorhabditis longicaudata (Bastian, 1865) Sudhaus, 2011	T	12
Crusthorhabditis	**C**	**31**
Cruznema	**C**	**31**
***Diploscapter coronatus* (Cobb, 1893) Cobb, 1913**	**Fw T C**	**31**
Mesorhabditis monhystera (Bütschli, 1873) Andrássy, 1976	T	1
Parasitorhabditis	**C**	**31**
***Pelodera teres* Schneider, 1866**	**C**	**31**
***Pelodera cylindrica* (Cobb, 1898) Dougherty, 1953**	**C**	**31**
Pelodera cystilarva (Völk, 1950) Dougherty, 1955	T	12
Pellioditis marina (Bastian, 1865) Andrássy, 1983	Bw (M)	1
Pellioditis pellioides (Bütschli, 1873) Andrássy, 1983	T	1
Poikilolaimus oxycerca (de Man, 1895) Sudhaus, 1980	T Fw	1

Protorhabditis filiformis (Bütschli, 1873) Sudhaus, 1976	T Fw	1
Protorhabditis oxyuroides Sudhaus, 1974	T	1
***Rhabditella axei* (Cobbold, 1884) Chitwood, 1933**	**T C**	**1**
Rhabditis terricola Dujardin, 1845	T	1
***Rhabditophanes cobbi* (Hnatewytsch, 1929) Andrássy, 1983**	**C**	**31**
Rhabditophanes schneideri (Bütschli, 1873) Goodey, 1953	T	12
Teratorhabditis	**C**	**31**

PART 2: *MONONCHOIDES COMPOSTICOLA* N. SP. (NEMATODA: DIPLOGASTERIDAE) ASSOCIATED WITH COMPOSTING PROCESSES: MORPHOLOGICAL, MOLECULAR AND AUTOECOLOGICAL CHARACTERIZATION.

3.6 Abstract

Mononchoides composticola n. sp. was isolated from compost and is described based on light and scanning electron microscopy, supplemented with SSU rDNA sequence data. It is characterised by the following features: a denticulate ridge in addition to the dorsal claw-like tooth, a small tooth-like swelling at the stegostom base, ca 26 longitudinal ridges on the female body, a uterine sac associated with two dumb-bell-shaped pouches, relatively small spicules (30-38 µm long), a simple gubernaculum shorter than half the spicule length, the genital subventral papillae (v6) consisting of three very small papillae, and a long filiform tail (female: 391-550 µm, 18-26 anal body diam.; male: 304-548 µm, 19-30 anal body diam.). Phylogenetic analyses placed the new species together with *M. striatus*, sister to *Tylopharynx foetida*. Since the use of nematodes as functional indicators often relies on the allocation of nematodes to feeding groups, experiments were performed to elucidate the feeding strategy of the new species. Both its ability to move actively to bacterial food sources and to prey on other compost nematodes were tested. *Mononchoides composticola* n. sp. actively moved towards the compost bacterium *Achromobacter*, a taxis that was temperature dependent, and also preyed on other nematodes. Predation was selective, with a higher predation rate on the relatively small and slow-moving *Rhabditella* sp. than on the considerably larger and more motile *Rhabditis* (*Poikilolaimus*) sp. Adults of *M. composticola* n. sp. have a dual feeding behaviour and can apparently alternate between bacterial and nematode prey.

3.7. Introduction

Until recently, knowledge of the nematode assemblages and population dynamics associated with composting processes was completely lacking. The nematode community in a Controlled Microbial Composting (CMC) process was analysed in Chapter 2. Compared to many soil ecosystems, nematode succession in compost differs mainly by the absence of K-strategists and by the prominence of diplogastrids. At the beginning of the composting process (thermophilic phase), immediately after the heat peak, the nematode population is primarily composed of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogasteridae) followed by bacterial-feeding (cp-2) (Cephalobidae) and fungal-feeding general opportunists (Aphelenchoididae). At the end of the process, at the most mature stage, the

fungal-feeding Anguinidae (mainly *Ditylenchus filimus* Anderson, 1983) were dominant. However, before this final stage, during the cooling and maturation stage, an unknown *Mononchoides* n. sp. became dominant (≥ 30% of nematodes). In view of the high abundance of this undescribed *Mononchoides* n. sp., it is reasonable to suggest that it may have a significant role in the compost-associated foodweb. *Mononchoides* Rahm, 1928 is a cosmopolitan, quite common, genus and by far the most diverse genus of the family Diplogasteridae *sensu lato* (Sudhaus and Fürst von Lieven 2003) (= taxon Diplogastromorpha in De Ley and Blaxter 2002). *Mononchoides* inhabits various terrestrial habitats and is often associated with compost, dung, mud, other decaying materials and with different kinds of beetles. Some *Mononchoides* species have been described from freshwater (e.g., sediment of rivers and lakes) and even marine habitats (e.g., sewage) (Sudhaus and Fürst von Lieven 2003). Within *Mononchoides*, Calaway and Tarjan (1973) listed 18, Andrássy (1984) 17 and Gagarin (1998) 24 species. Sudhaus and Fürst von Lieven (2003), following Gagarin (1998), considered *Glauximena* Allgén, 1947 synonymous with *Mononchoides*, an approach that is also followed in the current paper. In a comprehensive review on diplogastrid nematodes, also taking into account the detailed stoma morphology (see Fürst von Lieven and Sudhaus 2000), 43 species were listed within the genus *Mononchoides*. Andrássy (2005b) did not accept the synonymisation of *Mononchoides* and *Glauximena* based on the presence of a left ventro-sublateral plate in the buccal cavity of *Mononchoides* species. Andrássy (2005b) accepted only 29 valid *Mononchoides* species and nine valid *Glauxinema* species.

Since the review of Sudhaus and Fürst von Lieven (2003), *M. gaugleri* Siddiqi, Bilgrami & Tabassum, 2004 and *M. megaonchus* Mahamood, Ahmed & Shah, 2007 have been described while *M. tokobaevi* Lemzina, 1990 was not listed in Sudhaus and Fürst von Lieven (2003). Within *Glauxinema* (= synonym of *Mononchoides* according to Sudhaus and Fürst von Lieven, 2003), *G. aquaticum* Gagarin & Thanh, 2006 was recently described (Gagarin and Thanh 2006). Despite their high diversity and numerical importance, the exact feeding habits of *Mononchoides* species, and indeed of most diplogastrids, are barely known. In general they are considered bacterial-feeders and/or predators (Yeates et al., 1993). However, Fürst von Lieven and Sudhaus (2000) observed *Mononchoides* sp. feeding on fungal spores and ciliates. It is also very unclear which stages act as predators and which stages can switch to a bacterivorous mode when prey is absent. Knowledge of feeding type is important for our understanding of nematode ecology and for our use and interpretation of nematode-based indices used in community analyses and environmental monitoring (e.g., Index of Trophic Diversity; Heip et al. 1985).

In this paper we describe the new *Mononchoides* species isolated from compost based on morphology (studied by light microscopy (LM) and scanning electron microscopy (SEM)) and on molecular data (SSU rDNA sequences). Aspects of the feeding ecology of this new species are experimentally tested and the ecological implications of the observed feeding habits are considered.

3.8. Materials and methods

3.8.1. Collection and culture

Mononchoides composticola n. sp. was extracted from a compost heap at the Institute for Agricultural and Fisheries Research in Merelbeke near Ghent, Belgium (Plant Science Unit, Growth and Development research area), using a modified Baermann funnel method. The heap was composed of three different feedstock materials: 43% fine wood chips, 43% dry hay and 14% fresh grass. The compost was prepared according to the CMC method, but no microbial starter was added. A composting process is typically subdivided into three different phases based on the temperature profile: the thermophilic phase (45-75 °C), the cooling phase (45 °C - ambient temperature) and the maturation phase (≈ ambient temperature) (approximately 3, 3 and >10 weeks, respectively, in the composting process studied in Chapter 2). *Mononchoides composticola* n. sp. could be detected during the cooling and maturation phase from day 10 until day 165 of the process. A culture with bacteria (i.e., *Achromobacter* sp.) and nematodes (*Rhabditis* (*Poikilolaimus*) sp. and *Rhabditella* sp.) as food sources was established from one female and one male *M. composticola* n. sp.

Cultures were maintained on agar (bacterial agar 2.7 g 400 ml^{-1} (Oxoid, Basingstoke, UK)) and nutrient agar (1.3 g 400 ml^{-1} (Oxoid) plates containing cholesterol (final concentration of 1 µg ml^{-1} (Sigma-Aldrich, St Louis, MO, USA)). The cultures were kept in an incubator at 25 °C and generally handled as described by Brenner (1974).

The nutrient portion of the agar also stimulated the development of the natural bacteria present in the compost. An unknown compost bacterial strain was isolated and cultured in LB-medium (2 g yeast extract (Sigma Cell Culture), 4 g tryptone (Oxoid), 2 g NaCl (Acros Organics, Geel, Belgium), 0.4 g (1 M) NaOH and 400 ml distilled water, autoclaved and stored at 4 °C). Subsequently, the compost bacteria used for the feeding experiments were identified to genus level at the Laboratory of Microbiology, Faculty of Sciences, Ghent University. The bacteria used for the feeding experiments belonged to *Achromobacter*, a genus known from aquatic habitats and soil and which can also be found in clinical samples (Coenye et al. 2003).

3.8.2. Morphological characterisation

Type material was collected directly from modified Baermann funnel method extractions in a small drop of water in an embryo dish. Formalin (4% with 1% glycerin) was heated to 70 °C and an excess (4-5 ml) was quickly added to the specimens to fix and kill the nematodes instantly (Seinhorst 1966). The fixed nematodes were processed to anhydrous glycerin following the glycerin-ethanol method (Seinhorst 1959 as modified by De Grisse 1969). Measurements and drawings were prepared manually with a camera lucida on an Olympus BX51 DIC Microscope (Olympus Optical, Tokyo, Japan) equipped with an Olympus C5060Wz camera for photographs.

The holotype was also recorded as a video clip mimicking a multifocal observation through a LM microscope following the Video Capture and Editing procedures developed by De Ley and Bert (2002). The resulting virtual specimens are available at www.nematology.ugent.be/vce.html.

For SEM, specimens preserved in anhydrous glycerin were transferred to a drop of glycerin in an embryo dish. Two drops of water were added every 10 min for 2 h. A subsequent ultrasonic treatment (8 min) of the specimens in a single drop of water removed particles adhering to the body surface. The specimens were dehydrated by passing them through a graded ethanol concentration series of 20, 50, 75, 95, 100% (1 h each), 100% (overnight) and 100% (20 min, next morning). Afterwards, the specimens were critical point- dried with liquid CO_2, mounted on stubs with carbon discs and coated with gold (25 nm) before observation with a JSM-840 EM (JEOL, Tokyo, Japan) at 15 kV.

The terminology describing the parts of the stoma follows De Ley et al. (1995) and that of other structural details of the buccal cavity is in accordance with Fürst von Lieven and Sudhaus (2000), which is an essential paper for the correct interpretation of the morphological structures of the diplogastrid stoma region. The male genital papillae formula used was as proposed by Sudhaus and Fürst von Lieven (2003).

3.8.3. Molecular characterisation

For the molecular characterisation one specimen from each of two different cultures was used. One culture was started from *M. composticola* n. sp. specimens isolated on day 31 of the composting process using agar plates (isolate 'culture, day 31') and the other culture was started from *M. composticola* n. sp. specimens isolated on day 84 of the composting process using the modified Baermann funnel method (isolate 'direct extraction, day 84').

DNA extraction, PCR reaction and sequencing the SSU rDNA were done as in Bert et al. (2008). The sequences were deposited in GenBank under the accession numbers GU943511 and GU943512. Additional sequences of diplogastrids for phylogenetic analyses were obtained from GenBank. The SSU rDNA sequences were aligned with Clustal W (Thompson et al. 1994) and manually checked. This resulted in an alignment of 1679 characters of which 445 were

parsimony-informative. Differences between sequences were counted using the BioEdit sequence alignment options (Hall 1999). Bayesian phylogenetic inference (BI) was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). A general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + G) was used, as estimated by PAUP*/MrModeltest 2.0b (Nylander 2004). Two independent, simultaneous analyses were run for 3×10^6 generations and the trees were generated using the last 10^6 generations, well beyond the burn-in value and the point of convergence between the two runs, the latter confirmed by the average standard deviations of split frequencies which approached zero (<0.0012). Other analyses (maximum parsimony, maximum likelihood and LogDet-transformed distance) were done but did not alter the tree topology (except for the support values) and are not further discussed.

3.8.4. Feeding experiments

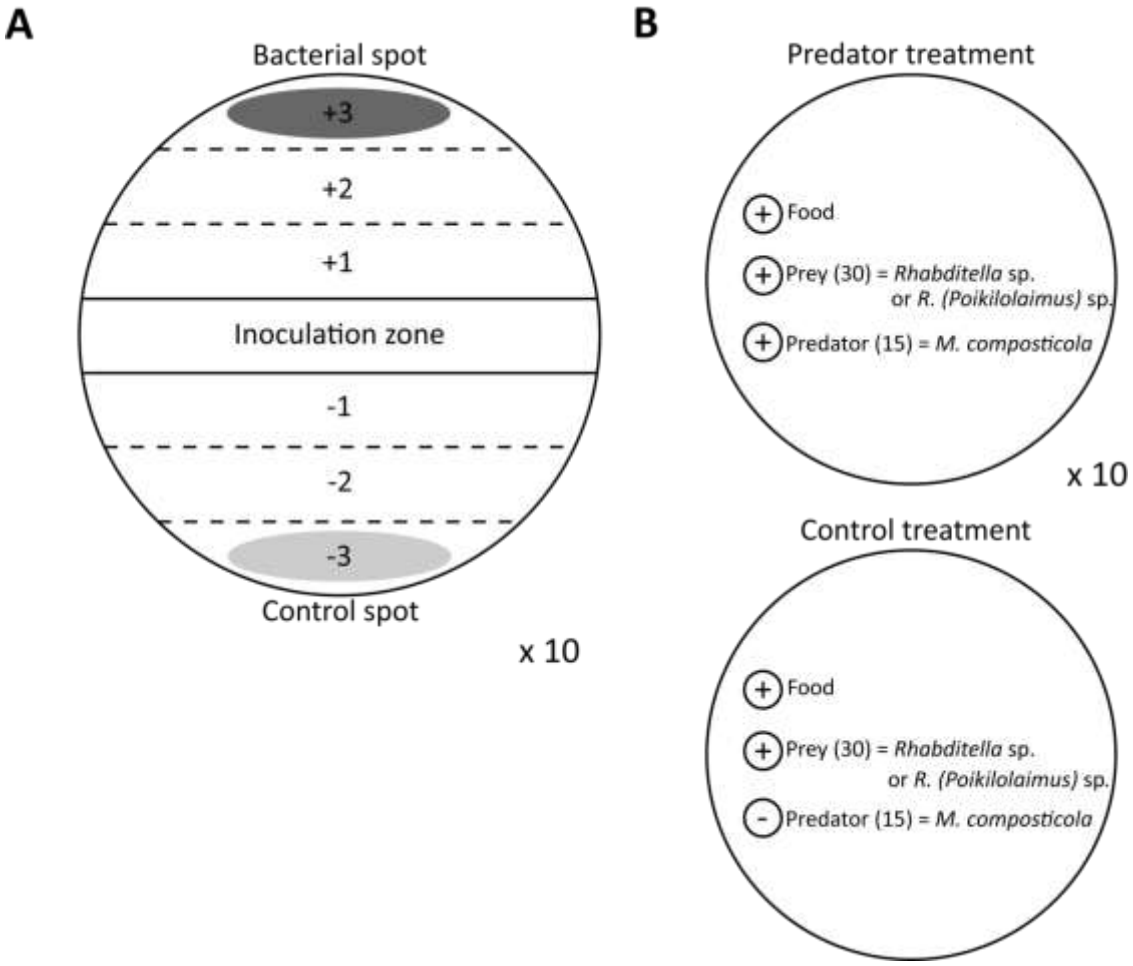
Preliminary tests were done to identify the best medium and temperature for successful culturing of *M. composticola* n. sp. Two kinds of feeding/foraging experiments were performed, assessing, respectively, the ability of *M. composticola* n. sp. to detect and actively move to a bacterial food source and its potential to feed as a predator on other compost nematodes. These experiments were born out of expectations based on literature information documenting, or at least suggesting, predatory feeding and bacterivory in other diplogastrid nematodes (Yeates et al. 1993; Moens et al. 2004).

To test directional active movement to bacterial food, a Petri dish (8.7 cm diam.) with a 1% agar layer (bacterial agar 4 g 400 ml^{-1} (Oxoid)) was subdivided into seven parallel regions, i.e., an inoculation zone with three positive regions to one side and three negative regions to the other. At the two most extreme, opposite sides (+3 and −3 region, respectively) of the Petri dish, either a 80 µl drop of the bacteria *Achromobacter* in LB medium (=bacterial spot) or a 80 µl drop of distilled water (= control spot) was placed (Figure 3.1A) Ten nematode specimens were washed in distilled water and added to the inoculation zone in the centre of the plate ca 1 h after spotting the candidate attractants. To emphasise the distance moved towards or away from the bacterial food source each nematode was scored: nematodes that had moved towards the bacterial attractant were awarded a positive score; the ones moving in the opposite direction were given a negative score, corresponding to the region in which they were recorded (so individual scores ranging from 0 to +3 and from 0 to −3 for migration towards or away from the bacterial spot, respectively). A Leica Mz95 binocular was used for observations. The movement towards or away from a bacterial food source was scored at 1, 2, 4 and 24 h after incubation. Once inside a bacterial spot, most nematodes tended to stay inside it. This experiment was repeated at six different temperatures (15, 20, 30, 40, 45 and 60 °C) with ten replicates of each,

always with ten *M. composticola* n. sp. per replicate. For the statistical analyses, only the data at 15, 20 and 30 °C were used because the nematodes did not survive the entire 24 h incubation at the higher three temperatures. Replicated G-tests for goodness of fit (Sokal and Rohlf 1995) were used to test the null hypothesis of random movement and hence no preferential migration of *M. composticola* n. sp. towards bacteria. This should result in a 1 : 1 ratio of nematode numbers inside bacterial and control spots. Differences in rate and degree of migration among different temperature treatments were analysed using repeated measures ANOVA followed by post hoc Tukey HSD tests for unequal number of replicates. These analyses were performed on an Attraction Index (Troemel et al. 1997) calculated for each replicate. In brief, this index sums the scores of each nematode on a plate and divides this sum by the number of nematodes recovered. It thus takes into account the position of all nematodes on a plate. The more positive the index, the closer, on average, nematodes on a plate were to the bacterial spot; the more negative the index, the closer, on average, nematodes were to the control spot.

Secondly, the ability of *M. composticola* n. sp. to feed on other compost nematodes was tested. The experiments were executed with two different candidate prey species, *Rhabditis* (*Poikilolaimus*) sp. and *Rhabditella* sp., with ten replicates of each. Petri dishes were filled with a nutrient agar layer covered with a layer of compost bacteria (70 µl bacteria in LB medium spread evenly). The predator treatments consisted of 15 adult *M. composticola* n. sp., 30 adult *R.* (*Poikilolaimus*) sp. or *Rhabditella* sp. and *Achromobacter* compost bacteria, while controls were similarly incubated but contained no *M. composticola* n. sp. (Figure 3.1B). Before inoculation on to the agar plates, nematodes were washed in distilled water to remove adhering debris. Prey and predator nematode numbers were counted after 24 and 48 h. Prey consumption was calculated as the difference between prey numbers remaining in the control and in the predator treatment (Moens et al. 2000). Any small juvenile prey nematodes produced during the experimental incubation were not taken into account for our calculations. The edges, walls and lids of the Petri dishes were checked to ensure that no prey had escaped from the agar layers. Natural mortality of both prey and predator was easily detectable since decay times of dead nematodes were longer than the incubation time of the experiments. The occurrence of empty prey cuticles was treated as removed prey. Statistical analysis of differences in the number of prey remaining after 24 and 48 h between the predator and control treatment used Student t -tests in Statistica 6.0. Differences in prey removal between the two different prey species after 24 h were also examined using a Student t -test.

Figure 3.1:
Schematic overview of the experimental setup of the feeding experiments performed with *Mononchoides composticola* n. sp. A: To test directional active movement to bacterial food, a Petri dish was subdivided into seven parallel regions (i.e. one inoculation zone, three posistive regions and three negative regions). The movement of the nematodes was scored 1, 2, 4 and 24 h after incubation and the experiment was repeated at 15, 20, 30, 40, 45 and 60°C in ten replicates. B: The ability of *M. composticola* to feed on other compost nematodes was tested by comparing the number of prey (*Rhabditella* sp. or *R. (Poikilolaimus)* sp.) in the predator treatment (including food, predators and prey) and the control treatment (including only food and prey).



3.9. Results

Mononchoides composticola[*] n. sp. (Figures 3.2-3.4)

3.9.1. Measurements
See Table 3.2.

3.9.2. Description
Female

Body long, straight to slightly arcuate ventrally after fixation. Cuticle clearly annulated, ca 1 µm at midbody, with conspicuous longitudinal ridges (ca 26 at head level). Lip region continuous with body contour, consisting of six fused lips, each with a small papilla. Stoma s.l. longer than

[*] The specific epithet refers to the type habitat of the new species.

wide. First stoma part or cheilostom (= stoma s.s.) wide, walls heavily cuticularised. Cheilostom subdivided into several (14-18) narrow, rod-like plates (= cheilorhabdions). Bifurcated apex of cheilorhabdions mostly extending beyond labial contour. Second part of stoma consisting of gymnostom and stegostom, both anisotopic with ventro-sublateral walls longer than dorsal. Relatively small elliptical aperture of amphid located at gymnostom level. According to Fürst von Lieven and Sudhaus (2000), inner wall of gymnostom bearing two dorsolateral denticles, although these could not be explicitly distinguished in this study. Anteriormost part of stegostom bearing a large, claw-like dorsal tooth, a right ventro-sublateral pyramidal hooked tooth and a left ventro-sublateral denticulate ridge. Claw-like dorsal tooth with tip pointing dorsally and with prominent duct of dorsal gland. Posterior part of stegostom (= meta- and telostegostom) forming a broad cylindrical tube (on average 4 µm diam. and 12 µm long). Stegostom cylinder on average three times longer than broad. Tooth-like swelling at base of stegostom. Neck region comprising ca 11-17% of body. Pharynx divided into muscular procorpus slightly expanding posteriorly into oval muscular metacorpus with valves and a short, non-muscular, glandular isthmus with non- muscular elongated oval basal bulb without valves. Corpus, isthmus and basal bulb in ratio 3 : 1 : 1. Nerve ring encircling isthmus in anterior half. Secretory-excretory pore more posterior (7-13 µm posterior to nerve ring) at transition of isthmus to basal bulb. Reproductive system amphidelphic with both branches equally developed, anterior branch on right, posterior branch on left side of intestine. Ovary long with oocytes arranged in one to two indistinct rows in ger- minal zone. Uterine sac ca 20 µm long, connecting both uteri. Vulval opening anterior to mid-body, barely visible as a small circular pore. Vulva lips weakly cuticularised, not protruding. Vagina muscular, with narrow lumen. Pair of dumb-bell-shaped pouches present at level of uterine sac. Phasmids prominent, located laterally posterior to anus. Tail long, filiform.

Male

General morphology similar to female but body slightly smaller, typically J-shaped after fixation. Anterior part with four additional cephalic setae situated on edge of lip region and elliptical amphidial aperture at gymnostom level. Denticulate ridge of different shape to that of female, but precise structure not unequivocal as seen by LM. Testis single, anteriorly ventrally reflexed. Spicules separate slightly arcuate. Gubernaculum with small distal sleeve and inconspicuously formed proximal apophysis. Nine pairs of genital papillae present: three pairs precloacal, six pairs postcloacal. Genital papillae formula: v1, v2, v3d/v4, ad, ph, (v5, v6(3), v7), pd. Papillae with subventral origin marked as ‘v1 to v7’, numbered from anterior to posterior. Papilla marked as ‘v3d’ originating laterally next to spicules, ‘v6(3)’ consisting of three very small papillae

grouped close together. Papillae marked as 'ad' (anterior dorsal papilla) and 'pd' (posterior dorsal papilla) originating subdorsally, ad located close to prominent phasmids (ph) and pd situated laterally at attenuating point of tail. Tail mostly shorter than female, filiform.

3.9.3. Type habitat and locality

Compost heap at the Institute for Agricultural and Fisheries Research in Merelbeke, Belgium (Plant Science Unit, Growth and Development research area); 50°59'16.62" N, 3°46'30.66" E; altitude: 18.6 m. During the composting process *M. composticola* n. sp. was present from day 10 until at least day 163. The heap was composed of three different feedstock materials: 43% (vol/vol) fine wood chips, 43% (vol/vol) dry hay and 14% (vol/vol) fresh grass.

3.9.4. Type material

Holotype female, four female paratypes and two male paratypes at the Museum voor Dierkunde (collection number UGMD 104180), Ghent University, Ghent, Belgium. Two female paratypes and two male paratypes at the Wageningen Nematode Collection (WT 3475), University and Research Centre, Landbouwhogeschool, Wageningen, The Netherlands.

3.9.5. Diagnosis and relationships

The most important traits to discriminate species within the genus *Mononchoides* are: (i) the shape and number of teeth or tooth-like structures in the stegostom, (ii) the length of the filiform tail, (iii) the position of the vulva, (iv) the length and shape of the spicules and gubernaculum and (v) the genital papillae formula. *Mononchoides composticola* n. sp. is characterised by a combination of the following morphological features: a denticulate ridge in addition to the dorsal claw-like tooth, a small tooth-like swelling at the stegostom base, ca 26 longitudinal ridges on the female body, a uterine sac associated with two dumb-bell-shaped pouches, spicules that are relatively small (30-38 µm), a simple gubernaculum less than half the spicule length long, the genital subventral papillae (v6) consisting of three very small papillae and an especially long filiform tail (female: 391-550 µm, 18-26 anal body diam.; male: 304-548 µm, 19-30 anal body diam.).

Mononchoides composticola n. sp. is most similar to the *Mononchoides* sp. described by Mahamood et al. (2007) but differs by having a longer tail (female: 391-550 vs 330-475 µm; male: 304-548 vs 204-369 µm), a longer female basal bulb (19-32 vs 16-20 µm), a shorter neck in the male (97-113 vs 118-143 µm), shorter and smaller spicules and gubernaculum (respectively 30-38 vs 37-43 µm and 10-14 vs 14-22 µm), the position of the female phasmids (1.4-1.9 vs 0.8-1.3 anal body diam. posterior to anus) and the 6th subventral genital papilla comprising three very small subpapillae vs a single papilla.

Mononchoides composticola n. sp. is also similar to the following species in having similar morphology and measurements: *M. andrassyi* (Timm, 1961) Gagarin, 1998; *M. flagellicaudatus*

Andrássy, 1962; *M. longicaudatus* Khera, 1965; *M. ruffoi* Zullini, 1981 and *M. parastriatus* Paesler, 1946. It differs from *M. andrassyi* by having a denticulate ridge in addition to the dorsal claw-like tooth and the smaller subventral tooth, a shorter female body length (810-1150 vs 1008-1420 µm), shorter spicules and gubernaculum (30-38 vs 36-42 µm and 10-14 vs 17-19 µm, respectively), a longer male tail (18-30 vs 12-16 anal body diam.) and by the different shape of the spicules (not cephalated) and shape of the gubernaculum; from *M. flagellicaudatus* by having a denticulate ridge, a larger stoma in the female (14-27 vs 13-14 µm), a shorter rectum (0.8-1.5 vs 1.8 anal body diam.), a shorter vulva-anus distance (less than two-thirds of tail length), a shorter gubernaculum (10-14 vs 15-16 µm), a higher tail/anal body diam. ratio in the male (19-30 vs 13-16) and by the lack of the hooked proximal end of the gubernaculum; from *M. longicaudatus* by having a shorter female body (810-1150 vs 1100-1400 µm), a shorter female isthmus (13-28 vs 48 µm), a shorter male procorpus (34-45 vs 62 µm), longer spicules (30-38 vs 23-25) without cephalated heads and most obviously by the shape of the dorsal tooth which is not tripartite; from *M. ruffoi* by the lack of an additional serrate cuticularised ring lining the base of the cheilostom and by having a shorter gubernaculum (10-14 vs 14-18 µm), more genital papillae (9 vs 7 pairs) and a longer male tail (304-548 vs 270-310 µm); and from *M. parastriatus* by having shorter and less heavily cuticularised cheilorhabdions, a lower c and b ratio in the male (1.9-2.8 vs 2-4 and 4.9-6.7 vs 6-8, respectively) and the lack of a pointed distal end to the gubernaculum. *Mononchoides composticola* n. sp. is relatively similar to *M. andersoni* Ebsary, 1986 but differs by having a longer metacorpus (101-115 vs 17-34 µm), a shorter stegostom cylinder (10-12 vs 18-25 µm), two subventral teeth at the stegostom base and by the presence of males. It also resembles *M. paramonovi* Gagarin, 1998; *M. pulcher* Zullini, 1981 and *M. vulgaris* Gagarin, 2000 morphometrically but lacks the small onchium at the base of the stegostom cylinder. *Mononchoides composticola* n. sp. has a sister relation to *M. striatus* (Bütschli, 1876) Goodey, 1963 based on the limited molecular data but differs by having a lower c ratio in females (1.9-2.8 vs 5.2-6.5), shorter spicules and gubernaculum (30-38 vs 40-48 µm and 10-14 vs 24-26 µm, respectively) and the 6th subventral genital papilla comprising three very small subpapillae vs a single papilla.

3.9.6. Phylogenetic analyses

The phylogenetic analysis (Figure 3.5) places the two compost isolates of *Mononchoides composticola* n. sp. together with maximal support, sister to *Mononchoides striatus*. The monophyletic *Mononchoides* clade has a maximally supported sister relation with *Tylopharynx foetida* (Bütschli, 1874) Goodey, 1928. The two isolates of *M. composticola* n. sp. only differ in two nucleotides (0.1%) from each other, while they differ in 15 nucleotides (0.9%) from the *M. striatus* sequence obtained from GenBank. The *M. composticola* n. sp. clade and *M. striatus*

(however, only represented by one sequence) were both supported by five autapomorphies (Figure 3.5).

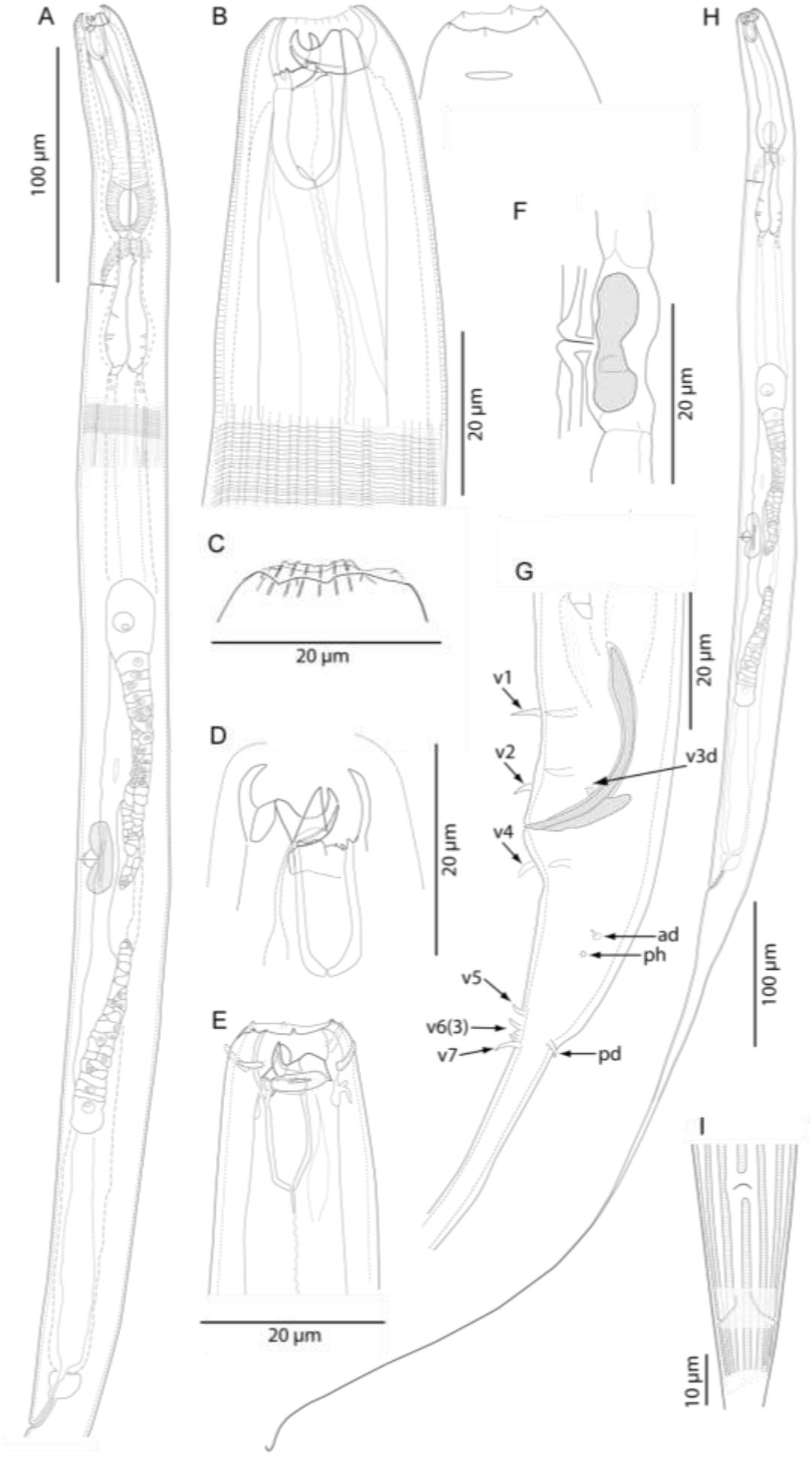
3.9.7. Feeding experiments

The ratio of *M. composticola* n. sp. reaching the bacterial and control spots after 24 h differed significantly from 1 : 1 at 15, 20 and 30 °C ($P < 0.05$), proving the attractiveness of this bacterial strain to the nematode. The positive response to bacteria, when taking into account all nematodes in the plate (Attraction Index), was most pronounced at 20 °C and the response showed a significant difference between the temperature treatments of 15 and 30 °C ($P = 0.02$). On average 3.86 ± 1.35, 4.8 ± 1.81 and 6.25 ± 2.3 out of ten *M. composticola* n. sp. moved into the bacterial spot after 24 h at 15 °C, 20 °C and 30 °C, respectively, compared to an average 2.71 ± 2.30, 0.4 ± 0.84 and 2.17 ± 1.9 *M. composticola* n. sp. in the control spot.

Figure 3.6 shows the change in prey numbers over a 2-day period in the presence (predator treatment) and absence (control treatment) of predators. On average, 50 and 80% of prey specimens (i.e., *R.* (*Poikilolaimus*) sp. and *Rhabditella* sp., respectively) were caught in the first 24 h and on average 70 and 92% prey (i.e., *R.* (*Poikilolaimus*) sp. and *Rhabditella* sp., respectively) had been removed after the full 48 h incubation, compared to 18 and 23% of *R.* (*Poikilolaimus*) sp. and of *Rhabditella* sp., respectively, that disappeared in the controls without predators after 48 h. Control and predator treatments showed significant differences ($P < 0.005$) in prey numbers remaining after 24 and after 48 h with both prey species. Over the first 24 h, each *M. composticola* n. sp. consumed on average 1.2 ± 0.3 *R.* (*Poikilolaimus*) sp. and 2 ± 0.7 *Rhabditella* sp. During the next 24 h, *M. composticola* consumed on average 0.9 ± 0.5 *R.* (*Poikilolaimus*) sp. and 1.1 ± 0.3 *Rhabditella* sp. After 24 h, the predation rate of *M. composticola* n. sp. on *Rhabditella* sp. was significantly higher compared to *R.* (*Poikilolaimus*) sp. ($P = 0.02$).

Figure 3.2:

Mononchoides composticola n. sp. from compost. A: Female (holotype) with neck region and reproductive system (without tail); B: Female head region: stoma *s.l.* and first part of procorpus with transverse striations and longitudinal ridges on cuticle plus superficial view of amphidial aperture; C: Rod-like plates (= cheilorhabdions) in cheilostom; D: Detail of female stegostom (lateral view) with dorsal claw-like tooth with prominent duct of dorsal gland, ventro-sublateral right pyramidal hooked tooth and left ventro-sublateral denticulate ridge; E: Male head region: stoma *s.l.* and cephalic setae and amphidial aperture; F: Detail of vulva and dumb-bell-shaped pouches at level of uterine sac; G: Detail of male posterior end with separate spicules, gubernaculum and nine pairs of genital papillae (v1, v2, v4, v5 and v7: subventral papillae. v3d: lateral papilla. v6(3): 6th subventral papilla consists of three very small papillae grouped close together. ad: anterior dorsal papilla. pd: posterior dorsal papilla. ph: phasmid); H: Habitus female (holotype); I: Female anus and phasmids (ventral view).


A
100 µm
B
20 µm
C
20 µm
D
20 µm
E
20 µm
F
20 µm
G
20 µm
v1
v2
v3d
v4
ad
ph
v5
v6(3)
v7
pd
H
100 µm
I
10 µm

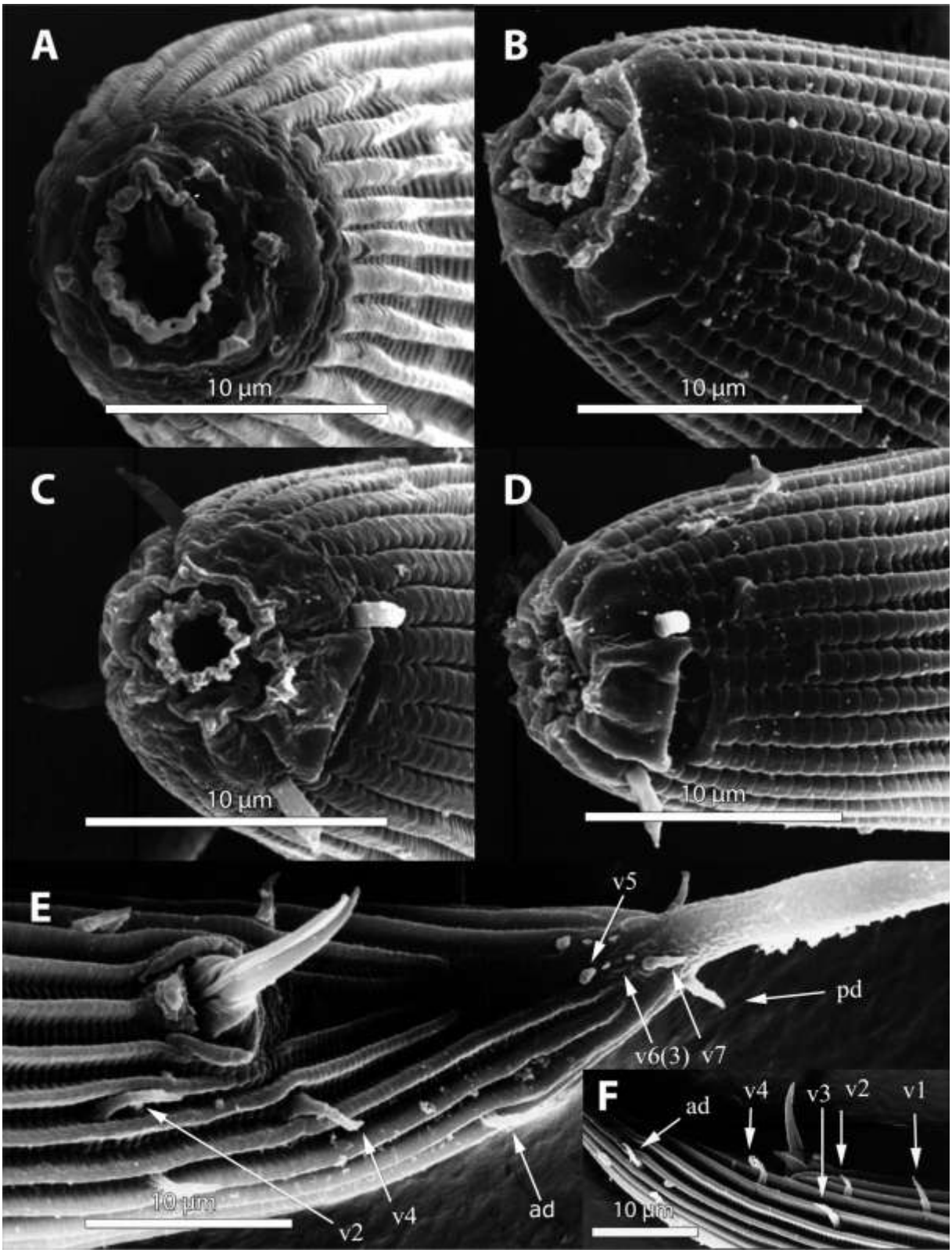


Figure 3.3:

SEM photographs of *Mononchoides composticola* n. sp. from compost. A: Female head region showing six fused lips, each with small papilla; B: Elliptical aperture of female amphid; C: Male lip region with four cephalic setae; D: Elliptical aperture of male amphid; E, F: Caudal genital papillae. Abbreviations as in Figure 3.2.

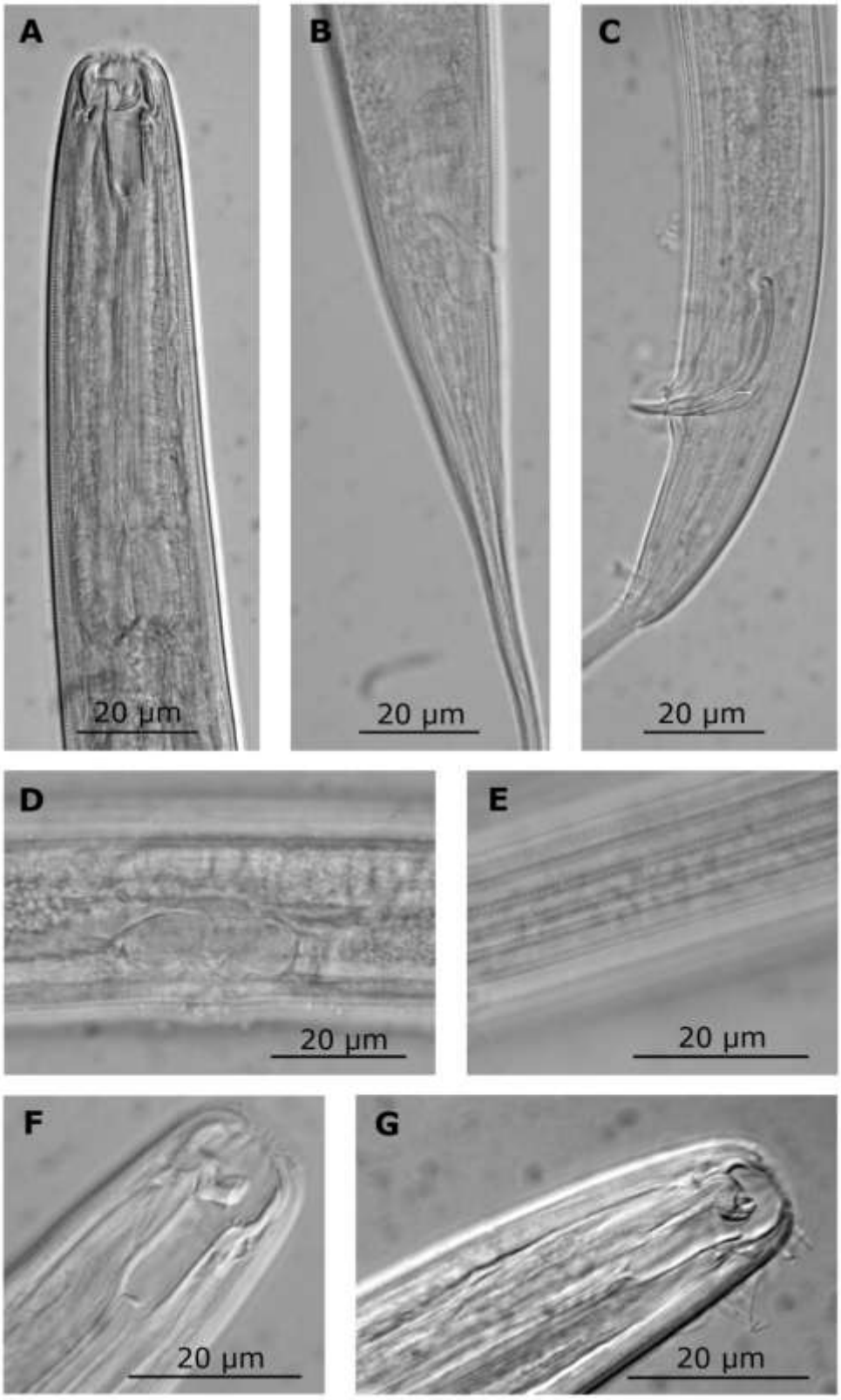


Figure 3.4:

LM Photographs of *Mononchoides composticola* n. sp. from compost. A: Female buccal cavity and first part of pharynx; B: Posterior end of female with anus; C: Posterior end of male with spicules and gubernaculum; D: Dumb-bell-shaped pouches at level of uterine sac; E: Annulated cuticle with longitudinal ridges; F: Detail of female stoma with denticulate ridge; G: Detail of female stoma with right pyramidal ventro-sublateral tooth and claw-like dorsal tooth with prominent duct of dorsal gland.

Table 3.2:
Morphometrics of *Mononchoides composticola* n. sp. from compost. All measurements are in µm and in the form: mean ± s.d. (range) CV.

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	-	30	16
L	1148	1028 ± 105 (810-1150) 10.2	830 ± 88 (761-1029) 10.6
L'	602	555 ± 100 (377-696) 17.9	441 ± 55 (382-572) 12.5
a	31.9	29.8 ± 3.6 (24-36) 12	31.2 ± 4.6 (26.3-40.4) 14.9
b	7.3	7.9 ± 0.9 (5.8-9.4) 12.5	5.6 ± 0.7 (4.9-6.7) 12.7
c	2.1	2.2 ± 0.3 (1.9-2.8) 11.7	2.2 ± 0.3 (1.9-2.8) 13.7
c'	25.4	23.5 ± 2.5 (18.5-26.3) 10.4	21.3 ± 3.8 (18.7-30.5) 17.8
V	33	33.1 ± 3.9 (26.7-40.1) 12	-
V'	63	62 ± 13 (44-86) 20.5	-
Max. body diam.	35.9	34.6 ± 3.6 (30-44) 10.4	25.9 ± 3.2 (16-30) 12.5
Stoma length	22	20 ± 2.4 (14.5-27.3) 12	16.8 ± 2 (13.3-20.9) 12.2
Head diam. (at amphid)	17.4	19 ± 1.5 (16.2-22.6) 8	15 ± 1.6 (13-17.4) 10.8
Stoma length/head diam.	1.3	1 ± 0.1 (0.8-1.3) 9.7	1.1 ± 01 (0.9-1.3) 8.8
Amphid width	5.8	6.1 ± 0.9 (5.2-7.5) 14.4	5.3 ± 1.4 (3-8.7) 25.8
Stegostom length	12.2	11.6 ± 1.6 (9.3-16.2) 14.3	10.3 ± 1.3 (8.7-13.9) 13
Stegostom width	5.2	4.0 ± 0.7 (2.9-5.8) 20	3.3 ± 0.6 (3-4.6) 16.8
Cheilostom width	8.7	7.5 ± 1.4 (6-11) 18.2	5.8 ± 1 (5-7) 17.2
Neck length	154	134 ± 9 (117-157) 6.7	116 ± 8.4 (94-128) 7.3
Corpus length	100	90 ± 6 (79-103) 6.2	73 ± 6.8 (52-84) 9.4
Procorpus length	75	67 ± 6 (55-80) 8.4	41 ± 3 (34-45) 7.5
Metacorpus length	25	23 ± 4 (17-34) 17.6	16 ± 3 (14-21) 16.8
Isthmus length	27	20 ± 3 (13-28) 15.7	25 ± 5 (14-32) 17.5
Bulb length	27	24 ± 4 (19-32) 16.3	17 ± 3 (13-23) 16.2
Corpus/isthmus+bulb	1.8	2.2 ± 0.2 (1.7-2.7) 10.4	1.7 ± 0.3 (1.2-2.3) 14.5
Secretory -excretory pore position (EP)	122	109 ± 12* (89-121) 11	94 ± 6 (85-103) 6.2
Nerve ring position (NR)	107	98 ± 11* (83-113) 11.5	77 ± 6 (60-86) 8.3

Table 3.2 *(Continued)*

Character	Female		Male
	Holotype	Paratypes	Paratypes
EP (as % Neck length)	77	77 ± 4*	64 ± 4
		(71-81) 5.7	(60-73) 6
NR (as % Neck length)	68	69 ± 2*	52 ± 3
		(66-72) 3.2	(48-61) 5.8
Tail length	545	473 ± 40	389 ± 71
		(391-550) 8.5	(304-548) 18.3
Anal body diam. (ABD)	21.5	20.3 ± 2.2	17.3 ± 1.5
		(17.4-24.9) 10.8	(13.9-19.7) 9
Tail/ABD	25.4	23.7 ± 2.4	22.5 ± 4.4
		(18.5-26.4) 10.1	(18.7-30.5) 19.3
Anus to phasmid distance	32.5	26.6 ± 5.6*	20.7 ± 3
		(20-33) 21.2	(17-26) 14.4
Anus to phasmid/ABD diam.	1.5	1.5 ± 0.2*	1.2 ± 0.2
		(1.4-1.9) 11.4	(0.9-1.5) 17.4
Rectum length	23	22 ± 3	-
		(16-28) 13.2	
Rectum/ABD	1.1	1.1 ± 0.2	-
		(0.8-1.5) 15.7	
Tail/Rectum	23.5	20.9 ± 7.6	-
		(17.3-30.2) 17.3	
Branch length	249	205 ± 46	-
		(132-316) 22.3	
Posterior gonad	126	103 ± 28	-
		(60-178) 26.9	
Anterior gonad	123	102 ± 22	-
		(66-145) 21.2	
Vulva position	382	333 ± 32	-
		(288-392) 9.5	
Vulva body diam.	37.1	33 ± 4	-
		(25-39) 10.8	
Vulva to anus distance	262	227 ± 30	-
		(168-307) 13.3	
Seta length	-		4.1 ± 0.7
			(2.9-5.8) 18.3
Testis length	-		222 ± 30
			(175-274) 13.6
Spicule length	-		34 ± 1.9
			(30-38) 5.6
Spicule/ABD	-		2 ± 0.2
			(1.6-2.4) 10.2
Gubernaculum length	-		12 ± 1
			(10.4-13.9) 8.2
Gubernaculum/spicule	-		0.4 ± 0.02
			(0.3-0.4) 6.4

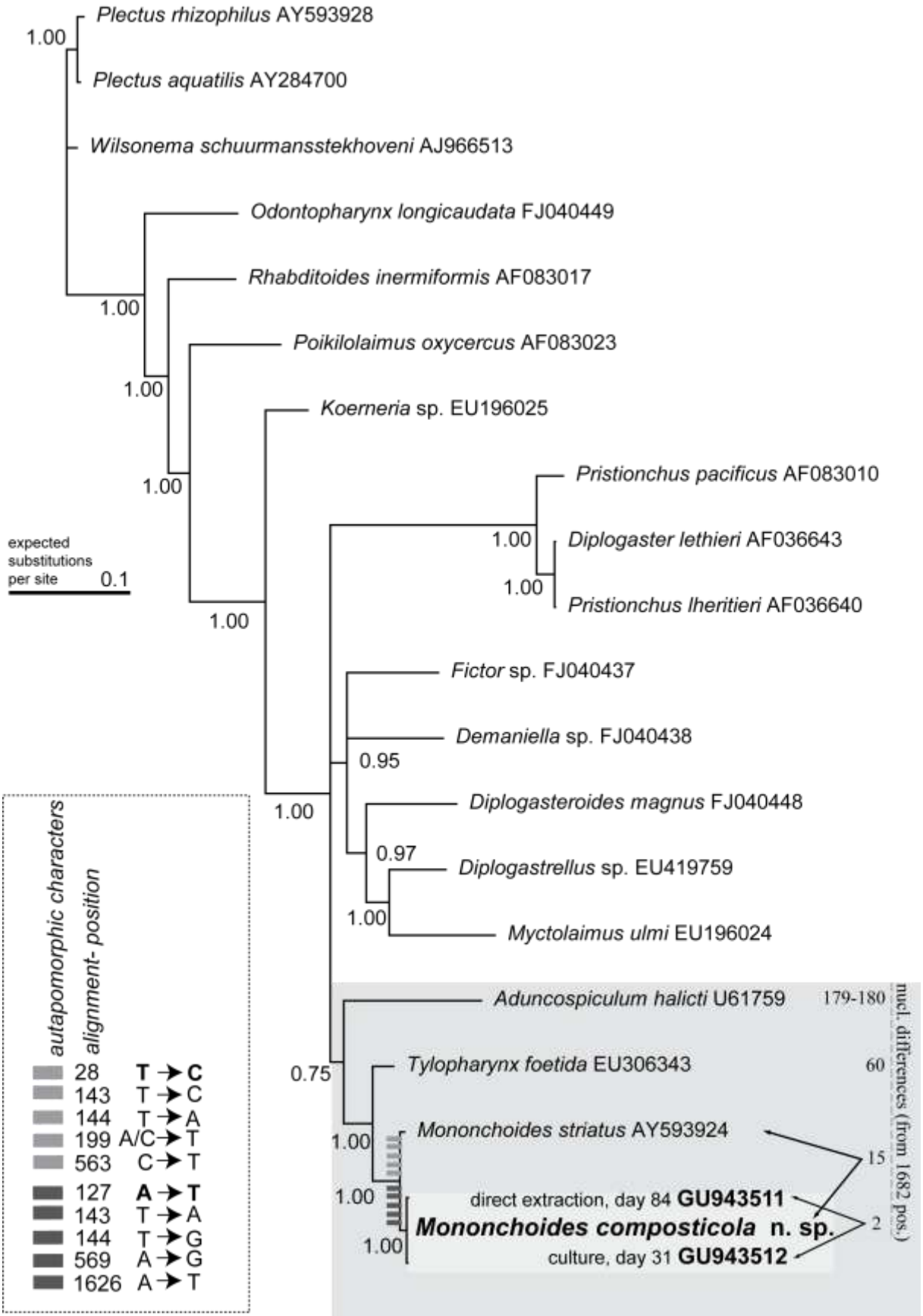

Plectus rhizophilus AY593928
Plectus aquatilis AY284700
Wilsonema schuurmansstekhoveni AJ966513
Odontopharynx longicaudata FJ040449
Rhabditoides inermiformis AF083017
Poikilolaimus oxycercus AF083023
Koerneria sp. EU196025
Pristionchus pacificus AF083010
Diplogaster lethieri AF036643
Pristionchus lheritieri AF036640
Fictor sp. FJ040437
Demaniella sp. FJ040438
Diplogasteroides magnus FJ040448
Diplogastrellus sp. EU419759
Myctolaimus ulmi EU196024
Aduncospiculum halicti U61759
Tylopharynx foetida EU306343
Mononchoides striatus AY593924
direct extraction, day 84 GU943511
Mononchoides composticola n. sp.
culture, day 31 GU943512
1.00
0.95
0.97
0.75
expected substitutions per site 0.1
autapomorphic characters
alignment- position
28 T → C
143 T → C
144 T → A
199 A/C → T
563 C → T
127 A → T
143 T → A
144 T → G
569 A → G
1626 A → T
179-180
60
15
2
nucl. differences (from 1682 pos.)

Figure 3.5:
Bayesian inference 50% majority rule consensus phylogeny of *Mononchoides composticola* n. sp. and other Diplogastromorpha sequences from GenBank based on SSU rDNA data. *Plectus rhizophilus, Plectus aquatilis* and *Wilsonema schuurmansstekhoveni* were designated as outgroup. Branch support values are indicated with Posterior Probability.

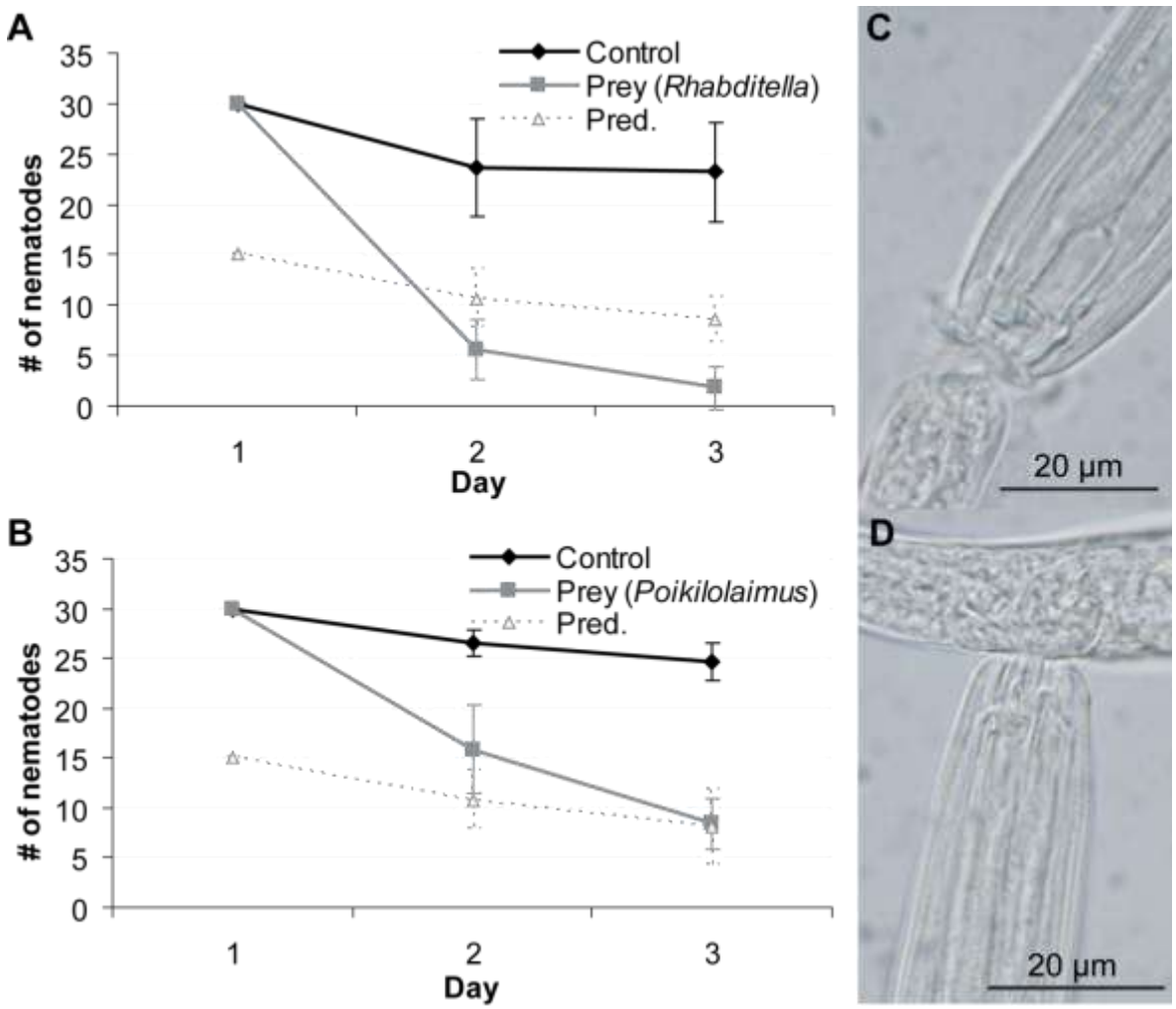


Figure 3.6:
Changes in prey and predator numbers over a 2-day incubation with two different prey species. Values are means ± s.d. of ten replicates per treatment. A: Prey = *Rhabditella* sp.; B: Prey = *Rhabditis* (*Poikilolaimus*) sp.; C, D: LM pictures of *Mononchoides composticola* n. sp. feeding on other compost nematodes.

3.10. Discussion

3.10.1. Phylogenetic position of *Mononchoides composticola* n . sp. and species characterisation

Our phylogenetic analysis indicates a sister relationship between *Mononchoides* and *Tylopharynx*, confirming the morphologically-based hypothesis that both genera are closely related (Figure 3.5). According to Fürst von Lieven and Sudhaus (2000), the right ventro-sublateral tooth was acquired in the ancestral line leading to both genera, whereas the stegostom cylinder and the firmly attached lateral tooth are an apomorphy for *Mononchoides* and *Tylopharynx*. The genus *Tylopharynx* figured prominently in the long lasting controversy over the origins and relationships of Tylenchomorpha (Maggenti 1963, 1983; Siddiqi 1980; Poinar 1983; Andrássy 1984 in Wu et al. 2001), especially because of the apparent similarity of its stoma armature to the tylenchid stylet. Based on the stoma structure of *T. foetida*, De Ley et al. (1993) suggested a different origin of the stoma of *Tylopharynx* and the stylet of *Tylenchida*. The common origin of *Tylopharynx* and *Mononchoides* stoma structures is now well supported, both by morphological and molecular data. The genus *Koerneria* Meyl, 1961 also has a stegostom cylinder, but according to Fürst von Lieven and Sudhaus (2000) it is unclear whether it is homologous with the stegostom cylinder of *Mononchoides* and *Tylopharynx*. Our results indicate that the two structures have evolved independently from each other since *Koerneria* is well separated from *Mononchoides* and *Tylopharynx* in our phylogenetic analysis (Figure 3.5).

Within *Mononchoides*, five autapomorphies for *M. composticola* n. sp. as well as *M. striatus* provide evidence of lineage exclusivity for both species. Thus, the species status of *M. composticola* n. sp. is consistent with an amalgamation of evolutionary and phylogenetic species concepts according to Adams (1998). A set of discovery operations to minimize the risk of making systematic errors (Adams 1998; Nadler 2002) make this approach both theoretically sound and practically feasible, especially in nematode taxonomy. However, we have to bear in mind that there are currently too few *Mononchoides* sequences available to depict a complete phylogenetic framework for the genus.

Mononchoides is an excellent example of a genus where molecular data are welcome to complete the current morphology-based species descriptions. The morphometric variation in this genus is striking and is clearly influenced by the environment and habitat (e.g., Gagarin 1998), and in our analyses it turned out that most measurements and ratios have remarkably high coefficients of variation (CV) (mostly between 10 and 20). Evidently, morphometric data alone are insufficient to argue lineage exclusivity in *Mononchoides*. Besides the inherent problem with intraspecific variability, the genus suffers from inadequate descriptions of some species and suggested synonymisations by several authors. Therefore, it is possible that several of the valid names according to Sudhaus and Fürst von Lieven (2003) are actually synonyms of other taxa: for an update of this list with morphometric data, see http://www.nematology.ugent.be/vce.html. Description based on detailed morphological observations (LM as well as SEM), morphometric data from multiple

specimens, molecular data and easily accessible digital vouchers would render many dubious species identities more solid.

3.10.2. Feeding behaviour of *M. composticola* n. sp.

Under natural conditions, many diplogastrids are known to feed on bacteria in addition to preying on nematodes (Pillai and Taylor 1968; Yeates 1969; Bilgrami and Jairajpuri 1989; Yeates et al. 1993; Fürst von Lieven and Sudhaus 2000). Our results are in agreement with this, since *M. composticola* n. sp. detected and migrated to bacterial colonies and preyed upon bacterial-feeding nematodes. Several *Mononchoides* species have been reported as predators on other nematodes: *M. potohikus* (Yeates, 1969), *M. longicaudatus* (Bilgrami and Jairajpuri, 1988), *M. fortidens* (Bilgrami and Jairajpuri, 1988), *M. gaugleri* (Bilgrami et al., 2005), *M. bollingeri* (Goodrich et al., 1968) and *M. changi* (Goodrich et al., 1968). As demonstrated in Figure 3.6C and D, and as described by Bilgrami and Jairajpuri (1989) and Fürst von Lieven and Sudhaus (2000), *Mononchoides* species pierce the cuticle of their prey by using their movable dorsal tooth, in addition to pharyngeal suction. They subsequently feed by ingesting some of the body contents of their prey. Our observations suggest that *Mononchoides* species do not always act as an ingester (as described by Yeates et al. 1993) but can also act as a piercer, as described in Bilgrami and Jairajpuri (1989) and Fürst von Lieven and Sudhaus (2000). *Mononchoides* sp. has also been reported feeding on large fungal spores (Fürst von Lieven and Sudhaus 2000), ciliates (Fürst von Lieven and Sudhaus 2000), oligochaetes (Small 1987), tardigrades (Small 1987), insect larvae (Small 1987) and amoebae (Small 1987). Both our experiments illustrate the dual feeding behaviour of adult *M. composticola* n. sp., which can apparently alternate between bacterial and nematode prey. The dual feeding behaviour hampers an unequivocal assignment of *M. composticola* n. sp. to a particular feeding type. Yeates et al. (1993) list the genus as bacterial-feeder (type 3) and predator (type 5a). In foodweb terminology, an organism feeding at more than one trophic level is an omnivore (Moens et al. 2004), yet the term omnivore also represents a feeding type (type 8) in the classification of Yeates et al. (1993), where it is not clearly defined. Its use appears to be restricted to certain dorylaimid nematodes. However, assigning a nematode to more than one feeding type (i.e., types 3 and 5a) hinders its inclusion in nematode-based environmental indices such as the enrichment and structure index or the index of trophic diversity.

Difficulties in maintaining *M. composticola* n. sp. in laboratory culture indicate that their high abundance during the composting process (Chapter 2) is intimately linked to aspects of their natural microhabitat in compost, involving a diversity of bacteria and nematodes and a particular temperature regime with temperatures up to 30 °C or more for several days. The better we mimicked such a compost environment, the higher the reproduction of *M. composticola* n. sp. (data not shown). Nevertheless, the results of the taxis experiments at different temperatures illustrate that the foraging efficiency of *M. composticola* n. sp. is maintained at temperatures down to 20 °C.

At still lower temperature (15 °C), food (bacteria) finding was significantly reduced. When food (bacteria and prey nematodes) availability was low, *M. composticola* n. sp. often exhibited cannibalism or died while searching for food, as already observed by Pillai and Taylor (1968).

The number of nematode prey decreased significantly after 24 and 48 h for both *R.* (*Poikilolaimus*) and *Rhabditella*. The per capita predation rates of *M. composticola* n. sp. on *R.* (*Poikilolaimus*) and *Rhabditella* after 24 h were 1.2 ± 0.3 and 2 ± 0.7, respectively. These rates are low compared to previous studies. Yeates (1969) reported a per capita predation rate of approximately 20 prey per 24 h for *M. colobocercus* (Andrássy, 1964) Sudhaus & Fürst von Lieven, 2003 feeding on *Bursilla littoralis* (Yeates, 1969) Andrássy, 1983, *Panagrolaimus australis* Yeates, 1969 and *Acrobeloides syrtisus* Yeates, 1967. *Mononchoides longicaudatus* and *M. fortidens* both consumed on average approximately 10 *Rhabditis* sp. in 24 h (Bilgrami et al. 2005). Predation rates can be influenced by prey density and by interference between different predators in the same habitat (Hamels et al. 2001). Yeates (1969), Bilgrami and Jairajpuri (1989) and Bilgrami et al. (2005) demonstrated that *Mononchoides* species show prey density-dependent predation rates.

Predation rates on *Rhabditella* sp. were significantly higher (at least during the first 24 h) than on *R.* (*Poikilolaimus*) sp. This may be related to prey size (on average 650 µm and 800 µm long, respectively) and/or motility. More motile prey have a higher encounter probability with predators (Moens et al., 2000), but may also have a better chance of escape upon a predator-prey encounter (Bilgrami and Jairajpuri 1989). In our experiment, *Rhabditella* sp. were considerably smaller and less motile than *R.* (*Poikilolaimus*) sp., the latter in fact exceeding *M. composticola* n. sp. in size, suggesting that it was less vulnerable to attack by *M. composticola* n. sp. Prey preference was also reported for *M. longicaudatus*, *M. fortidens* (Bilgrami and Jairajpuri 1988) and *M. gaugleri* (Bilgrami et al. 2005) and has been attributed to a variety of factors such as prey density, prey secretions, predator ability to wound prey, prey ability to avoid predation, and temperature.

The high abundance of *M. composticola* n. sp. during the composting process, its capacity to prey on other nematodes and its high foraging activity at temperatures below those typical of the compost microenvironment all suggest that the abundance of *M. composticola* n. sp. in maturing compost may be an important factor in determining the biocontrol potential of compost when applied to soils. The microclimate and abundant food sources in compost apparently render compost an ideal 'carrier medium' of *M. composticola* n. sp.

Acknowledgements

We thank Marjolein Couvreur for the SEM analyses and Dr. Vladimir Gagarin for his help with the search for original species descriptions. We are grateful to Prof. Dr. Anne Willems from the Laboratory of Microbiology, Faculty of Sciences, Ghent University for the identification of the

compost bacteria. Hanne Steel acknowledges the Foundation for Scientific Research, Flanders (FWO Vlaanderen, Belgium) for providing a grant as aspirant.

3.11. References

Adams, B.J., 1998. Species concepts and the evolutionary paradigm in modern nematology. J. Nematol. 30, 1-21.

Allgén, C.A., 1947. Weitere Untersuchungen über schwedische Nematoden. Kungl. Fysiogr. Sallsk. I Lund Forh. 7, 1-10.

Anderson, R.V., 1983. An emended description of *Ditylenchus valveus* Thorne & Malek, 1968 and description of *D. filimus* n. sp. (Nematoda: Tylenchidae) from mushroom compost in Canada. Can. J. Zool. 61, 2319-2323.

Andrássy, I., 1962. Neue Nematoden-Arten aus Ungarn I. Zehn neue Arten der Unterklasse Secernentea (Phasmidia). Acta Zool. Hung. 8, 1-23.

Andrássy, I., 1984. Bestimmungsbücher zur bodenfauna europas: Klasse Nematoda: (Ordnungen: Monhysterida, Desmoscolecida, Aeraeolaimida, Chromodorida, Rhabditida). Gustav Fischer Verlag, Stuttgart, pp. 228.

Andrássy, I., 1984. Klasse nematoda (Ordnungen Monhysterida, Desmoscolecida, Araeolaimida, Chromadorida, Rhabditida). Stuttgart, Germany, Gustav Fischer Verlag, 509 pp.

Andrássy, I., 2005a. Free-living nematodes of Hungary (Nematoda errantia): Volume I. Hungarian Natural History Museum. 518 pp.

Andrássy, I., 2005b. Free-living nematodes of Hungary, III (Nematoda errantia). In: Csuzdi, C., Zicsi, A. (Eds). Pedozoologica Hungarica: Taxonomic, zoogeographic and faunistic studies on the soil animals No. 3. Budapest, Hungary, Hungarian Natural History Museum and Systematic Zoology Research Group of Hungarian Academy of Sciences, pp. 452-464.

Andrássy, I., 2007. Free-living nematodes of Hungary (Nematoda errantia): Volume II. Hungarian Natural History Museum. 608 pp.

Andrássy I., 2009. Free-living nematodes of Hungary (Nematoda errantia): Volume III. Hungarian Natural History Museum. 496 pp.

Artois, T., Fontaneto, D., Hummon, W.D., McInnes, S., Todaro, M.A., Sorensen, M.V., Zullini, A., 2011. Ubiquity of microscopic animals? Evidence from morphological approach in species indentification. In: Fontaneto,D. (Ed.)., Biogeography of microscopic organisms: is everything small everywhere? Cambridge University Press, New York, pp. 244-284.

Athari, A., Mahmoudi, M.R., 2008. *Diploscapter coronatus* infection in Iran: report of the first case and review of literature. Iranian J. Parasitol. 3, 42-47.

Bert, W., Geraert, E., 2000. Nematode species of the order Tylenchida, new to the Belgian nematofauna with additional morphological data. Belg. J. Zool. 130, 47-57.

Bert, W., Coomans, A., Claerbout, F., Geraert, E., Borgonie, G., 2003. Tylenchomorpha (Nematoda: Tylenchida) in Belgium, an updated list. Nematology 5, 35-440.

Bert, W., Messiaen, M., Hendrickx, F., Manhout, J., De Bie, T., Borgonie, G., 2007. Nematode communities of small farmland ponds. Hydrobiologia 583, 91-105.

Bert, W., Leliaert, F., Vierstraete, A.R., Vanfleteren, J.R., Borgonie, G., 2008. Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nematoda: Rhabditida). Mol. Phylogenet. Evol. 48, 728-744.

Bert, W., Manhout, J., Van Colen, C., Borgonie, G., Decraemer, W., 2009. Nematode assemblages in a nature reserve with historical pollution. Belg. J. Zool. 139, 3-14.

Bilgrami, A.L., Jairajpuri, M.S., 1988. Attraction of *Mononchoides longicaudatus* and *M. fortidens* (Nematoda: Diplogasterida) towards prey and factors influencing attraction. Rev. Némat. 11, 195-202.

Bilgrami, A.L., Jairajpuri, M.S., 1989. Predatory abilities of *Mononchoides longicaudatus* and *Mononchoides fortidens* (Nematoda, Diplogasterida) and factors influencing predation. Nematologica 35, 475-488.

Bilgrami, A.L., Gaugler, R., Brey, C., 2005. Prey preference and feeding behaviour of the diplogastrid predator *Mononchoides gaugleri* (Nematoda: Diplogastrida). Nematology 7, 333-342.

Bongers, T.,1994. De nematoden van Nederland. Koninklijke Nederlandse Natuurhistorische Vereniging, Utrecht, The Netherlands, 408pp.

Brenner, S., 1974. Genetics of *Caenorhabditis elegans*. Genetics 77, 71-94.

Bütschli , O., 1876. Untersuchungen über freilebende Nematoden und die Gattung *Chaetonotus*. Z. Wiss. Zool. 26, 363-413.

Calaway, W.T., Tarjan, A.C., 1973. A compendium of the genus *Mononchoides* Rahm, 1928 (Diplogastrinae: Nematoda). J. Nematol. 5, 107-116.

Coenye, T., Vancanneyt, M., Falsen, E., Swings, J., Vandamme, P., 2003. *Achromobacter isolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. Int. J. Syst. Evol. Micr. 53, 1819-1824.

Coomans, A., 1989. Overzicht van de vrijlevende nematofauna van België (Nematoda). In: Proceedings of the symposium "Invertebraten van België". Brussels, Belgium K.B.I.N., 25-26 November 1988. Royal Belgian Institute of Natural Sciences, pp. 43-56.

Coosemans, J., 2002. Nematoden als indicators voor bodemclassificatie. *In:* Peeters, M., Van Goethem, J.L. (Eds). Proceedings of the symposium "Status and trends of the Belgian fauna with a particular emphasis on alien species". Brussels, Belgium K.B.I.N. Bulletin van het Koningklijk Belgisch Instituut voor Natuurwetenschappen Biologie 72, pp. 51-62.

De Grisse, A.T., 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. Meded. Fac. Landbouwwet. Gent 34, 351-369.

De Ley, P., De Grisse, A.T., Van De Velde, M.C., Coomans, A., 1993. Ultrastructure of the stoma in *Tylopharynx*. Meded. Fac. Landbouwwet. Gent 58, 763-777.

De Ley, P., Van De Velde, M.C., Mounport, D., BauJard, P., Coomans, A., 1995. Ultrastructure of the stoma in Cephalobidae, Panagrolaimidae and Rhabditidae, with a proposal for a revised stoma terminology in Rhabditida (Nematoda). Nematologica 41, 153-182.

De Ley, P., Bert, W., 2002. Video capture and editing as a tool for the storage, distribution, and illustration of morphological characters of nematodes. J. Nematol. 34, 296-302.

De Ley, P., Blaxter, M., 2002. Systematic position and phylogeny. In: Lee, D.L. (Ed.). The biology of nematodes. London, UK, Taylor & Francis, pp. 1-30.

Ebsary, B.A., 1986. *Mononchoides andersonin*. sp. and two new species of *Koerneria* (Nematoda: Diplogasteridae). Can. J. Zool. 64, 2012-2020.

Fonderie, P., DeVries, C., Verryken, K., Ducatelle, R., Moens, T., van Loon, G., Bert, W. Maxillary granulomatous inflammation caused by Halicephalobus gingivalis (Nematoda) in a Connemara mare in Belgium. Journal of equine veterinary science, in press. (http://dx.doi.org/10.1016/j.jevs.2012.05.066)

Fürst Von Lieven, A., Sudhaus, W., 2000. Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. J. Zool. Syst. Evol. Res. 38, 37-63.

Gagarin, V.G., 1998. A review of the genus *Mononchoides* Rahm, 1928, with description of two new species from European Russia (Nematoda: Diplogasterida). Zoosyst. Rossica 7, 229-238.

Gagarin, V.G., 2000. Nematode fauna of manure and compost in Yaroslavl oblast (Russia). Zool. Zh. 79, 1260-1274.

Gagarin, V.G., Thanh, N.V., 2006. Re-classification of Neodiplogasteridae with notes on the genus *Glauxinema* Allgén, 1947 and description of *G. aquaticum* sp. n. from Vietnam (Nematoda). Zoosyst. Rossica 15, 1-6.

Geraert, E., Sudhaus, W., Lenaerts, L., Bosmans, E., 1988. *Halicephalobus laticauda* sp.n., a nematode found in a Belgian coal mine (Nematoda, Rhabditida). Ann.Soc. Roy. Zool. Bel. 118, 5-12.

Geraert, E., 2010. The Criconematidae of the World. Identification of the Family Criconematidae (Nematoda). Ghent, Academia Press, 615 pp.

Goodrich, M., Hechler, H.C., Taylor, D.P., 1968. *Mononchoides changin*. sp. and *M. bollingerin*. sp. (Nematoda: Diplogasterinae) from a waste treatment plant. Nematologica 14, 25-36.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95-98.

Hamels, I., Moens, T., Muylaert, K., Vyverman, W., 2001. Trophic interactions between ciliates and nematodes from and intertidal flat. Aquat. Microb. Ecol. 26, 61-72.

Heip, H.R.C., Vincx , M., Vranken, G., 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Annu. Rev. 23, 399-489.

Hodda, M., Peters, L., Traunspurger, W., 2009. Nematode Diversity in Terrestrial, Freshwater Aquatic and Marine Systems. In: Wilson,M.J., Kakouli-Duarte,T. (Eds.), Nematodes as Environmental Indicators. CABI, Cambridge, UK, pp. 45-93.

Hunt, D.J., 1993. Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics. CAB International, Wallingford, 352 p.

Karssen, G., Waeyenberge, L., Moens, M., 2000. *Pratylenchus brzeskii* sp.nov. (Nematoda: Pratylenchidae), a root-lesion nematode from European coastal dunes. Ann. Zool. 50, 255-261.

Khera, S., 1965. Nematodes from the banks of still and running waters. I. *Tridontus longicaudatus* n. g., n. sp., subfamily Diplogasterinae Micoletzky, 1922 from India. Nematologica 11, 249-254.

Kovaleva, E.S., Subbotin, S.A., Masler, E.P., Chitwood, D.J., 2005. Molecular characterization of the actin gene from cyst nematodes in comparison with those from other nematodes. Comp. Parasitol. 72, 39-49.

Lemzina, L.V., 1990. Nematodes of Sary-Chelek lake. Izv. Akad. Nauk Kirg. SSR 3, 61-65.

Leroy, B.L.M., De Sutter, N., Ferris, H., Moens, M., Reheul, D., 2009. Short-term nematode population dynamics as influenced by the qaulity of exogenous organic matter. Nematology 11, 23-38.

Madan, M., Subbotin, S.A., Moens, M., 2005. Quantitative detection of the potato cyst nematode, *Globodera pallida*, and the beet cyst nematode, *Heterodera schachtii*, using Real-Time PCR with SYBR green I day. Mol Cell. Probe. 19, 81-86.

Mahamood, M., Ahmad, I., Shah, A.A., 2007. Nematodes of the order Rhabditidae from India. Description of two species of *Mononchoides* (Nematoda: Diplogastrina). J. Nemat. Morph. Syst. 10, 1-10.

Moens, T., Herman, P., Verbeeck, L., Steyaert, M., Vincx, M., 2000. Predation rates and prey selectivity in two predacious estuarine nematode species. Mar. Ecol. Prog. Ser. 205, 185-193.

Moens, T., Yeates, G.W., De Ley, P., 2004. Use of carbon and energy sources by nematodes. In: Cook, R., Hunt, D.J. (Eds). Proceedings of the Fourth Congress of Nematology, June 2002, Tenerife, Spain. Nematology Monographs and Perspectives 2. Leiden, The Netherlands, Brill, pp. 529-545.

Nadler, S.A., 2002. Species delimitation and nematode biodiversity: phylogenies rule. Nematology 4, 615-625.

Nadler, S. A., Carreno, R. A., Adams, B. J., Kinde, H., Baldwin, J. G., Mundo-Ocampo, M., 2003. Molecular phylogenetics and diagnosis of soil and clinical isolates of *Halicephalobus gingivalis* (Nematoda : Cephalobina : Panagrolaimoidea), an opportunistic pathogen of horses. Int. J. Parasitol 33, 1115-1125.

Nylander, J.A.A., 2004. MrModeltest v2. Uppsala, Evolutionary Biology Centre, Uppsala University.

Paesler, F., 1946. Beitrag zur Kenntnis der im Dünger lebenden Nematoden. Öst. Zool. Zeitschr. 1, 114-116.

Pillai, J.K., Taylor, D.P., 1968. Biology of *Paroigolaimella bernensis* and *Fictor anchicoprophaga* (Diplogasterinae) in laboratory culture. Nematologica 14, 159-169.

Rahm, G., 1928. Alguns nematodes parasitas e semi-parasitas das plantas culturaes do Brasil. Archives Institute of Biology, São Paulo 1, 239-252.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572-1574.

Rubtsova, T.V., Subbotin, S.A., Brown, D.J.F., Moens, M., 2001. Description of Longidorus sturhani sp. n. (Nematoda: Longidoridae) and molecular characterisation of several longidorid species from Western Europe. Russ. J. Nematol. 9, 127-136.

Sabu, L., Devada, K., Subramanian, H., Cheeran, J.V., 2002. *Diploscapter coronata* (Cobb, 1893) infestation in captive snakes. Zoos' Print Journal 12, 954-956.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4, 67-69.

Seinhorst, J.W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178.

Siddiqi, M.R., 2000. Tylenchida Parasites of Plants and Insects, 2nd edition. CABI Publishing, Wallingford, UK, 864 pp.

Siddiqi , M.R., Bilgrami, A.L., Tabassum, K.A., 2004. Description and biology of *Mononchoides gaugleri* sp. n. (Nematoda: Diplogastrida) from New Jersey, USA. International J. Nematol. 14, 129-134.

Small, R.W., 1987. A review of the prey of predatory soil nematodes. Pedobiologia 30, 179-206.

Sokal, R.R., Rohlf, F.J., 1995. Biometry, 3rd edition. New York, NY, USA, W.H. Freeman and Co., pp. 716-718.

Spiridonov, S.E., Moens, M., 1999. Two previously unreported species of steinernematids from woodlands in Belgium. Russ. J. Nematol. 7(1), 39-42.

Spiridonov, S.E., Reid, A.P., Podrucka, K., Subbotin, S.A., Moens, M., 2004. Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. Nematology 6, 547-566.

Sturhan, D., Hallmann, J., 2010. The genus *Hirschmanniella* (Tylenchida: Pratylenchidae) in Europe, with description of *H. halophila* sp. n. from Germany and notes on *H. caudacrena*. Nematology 12, 809-826.

Subbotin, S.A., Sturhan, D., Rumpenhorst, H.J., Moens, M., 2003. Molecular and morphological characterisation of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Nematology 5, 515-538.

Subbotin, S.A., Waeyenberge, L., Moens, M., 2000. Identification of cyst forming nematodes of genus Heterodera (Nematoda: Heteroderidae) based on the ribosomal DNA-RFLP. Nematology 2 , 153-164.

Sudhaus, W., Fürst Von Lieven, A., 2003. A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda). J. Nemat. Morph. Syst. 6, 43-90.

Sudhaus, W., Fitch, D., 2001. Comparative Studies on the Phylogeny and Systematics of the Rhabditidae (Nematoda). J.Nematol.33, 1-70.

Thompson, J.D., Higgins , D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positionspecific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-4680.

Tiasi, J., Chatterjee, A., Manna, B., 2010. Two new predatory species (mononchida: nematoda) under the genus *Mylonchulus* from West Bengal, India with a revised key to the species under the genus *Mylonchulus*. Rec. Zool. Surv. India 110, 15-29.

Timm, R.W., 1961. Five species of *Diplogaster* (Nematoda, Diplogasteridae) from east Pakistan. Pakistan J. Biol. Agric. Sci. 4, 1-6.

Troemel, E.R., Kimmel, B.E., Bargmann, C.I., 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. Cell 91, 161-169.

Vandenbossche, B., Viaene, N., De Sutter, N., Maes, M., Karssens, G., Bert, W., 2011. Diversity and incidence of plant-parasitic nematodes in Belgian turf grass. Nematology 13, 245-256.

Viaene, N., Wiseborn, D.B., Karssen G., 2007. First report of the root-knot nematode *Meloidogyne minor* on turfgrass in Belgium. Plant Dis. 91, 908.

Wharton, D.A., 2004. Survival strategies. In: Gaugler,R., Bilgrami,A.L. (Eds.) Nematode Behaviour. CABI, Cambridge, UK, pp. 371-400.

Wobalem, W., 2004. Survey of plant-parasitic nematodes and their antagonists in greenhouse cultures of vegetables in Flanders, Belgium. Masterthesis, Faculty of Sciences, Ghent University

Wu, J.H., De Ley, P., Liang, Y.L., 2001. Description of a new species of the genus *Tylopharynx* (Nemata: Diplogasteroidea), *T. clariamphida* sp. n., with a redescription of *T. foetida* (Bütschli, 1874) Goffart, 1930. J. Nematol. 33, 83-90.

Yeates, G.W., 1969. Predation by *Mononchoides potohikus* (Nematoda-Diplogasteridae) in laboratory culture. Nematologica 15, 1-9.

Yeates, G.W., Bongers , T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera – an outline for soil ecologists. J. Nematol. 25, 315-331.

Zullini, A., 1981. Three new species of diplogastrids (Nematoda). Boll. Mus. Civ. Stor. Nat. Verona 7, 395-404.

4. The nematode community as a proxy of the microbial community in a rapidly changing compost environment

Modified from:

Steel H, Buchan D, De Neve S, Couvreur M, Moens T and Bert W. Nematode and microbial communities in a rapidly changing compost environment: how nematode assemblages reflect composting phases. Submitted to European Journal of Soil Biology.

4.1 ABSTRACT

The microbial community (fungi and bacteria) is the main decomposer of organic matter during composting. Its composition is sometimes used as a proxy to assess compost maturity. Although nematodes are probably its most important grazers, only one previous study has highlighted clear shifts in nematode species composition during composting, and the assumption that nematodes reflect changes in the microbial community in compost has not yet been formally tested. Here, the microbial and nematode communities of a single composting process are analyzed together for the first time. Although both displayed broadly similar patterns, the abundance of fungal-feeding nematodes showed a distinct delay as compared to the increase in fungal PLFA. We argue that the nematode community may be a more promising tool to use in assessing compost maturity because it allows to discriminate between the three composting phases. First, during the thermophilic phase, bacterial-feeding nematodes dominate; during cooling, the bacterial feeders/predators bloom; and during maturation, the abundance of fungal feeders increases. Based solely on the microbial community, it was only possible to discriminate between the thermophilic phase and the rest of the process. Bacteria dominated during the thermophilic phase, while bacterial and fungal PLFA had more-or-less equal shares during cooling and maturation.

4.2 INTRODUCTION

Aerobic composting is the controlled biological oxidative decomposition of organic (waste) materials by successive communities of microorganisms under various temperature regimes, leading to the production of carbon dioxide (CO_2), water, minerals, and a humified end product (Butler et al. 2001). In nature, the mineralization of organic material is a slow process that does not produce a recognizable heat peak (Kutzner 2000). Composting mimics this natural process, but it does so under optimized environmental conditions (moisture levels, temperature, substrate composition, and oxygenation), resulting in a much faster process that yields a stable, high-quality end product (Zucconi and de Bertoldi 1987). The benefits of compost addition to the soil's physical and chemical characteristics include increased soil organic matter content (Termorshuizen et al. 2004) and the subsequent improvement of soil aeration, soil porosity, drainage, and water holding capacity (Cogger 2005). Furthermore, composts may have disease-suppressive properties that operate via a combination of physiochemical and biological mechanisms (Bailey and Lazarovits 2003; Oka 2010). Microbial and faunal communities in

compost shift continuously during composting. Eventually, at compost maturity, a more-or-less stable community of organisms becomes established (Ryckeboer et al. 2003; Chapter 2). An important question in compost research is how to identify reliable indicators of compost stability and maturity, which may be used to establish an optimal composting time. Such indicators may be chemical, physical, and/or biological in nature (Butler et al. 2001). Previously, numerous tests, based on both physical and chemical parameters, have been proposed (for a review, see Wichuk and McCartney (2010). However, none of these have been proven to be rigorous, reliable, or consistent enough to be used as a stand-alone maturity and stability measure, or they require such a substantial investment in laboratory equipment and staff training that their routine use is hampered (Wichuk and McCartney 2010).

Microorganisms play key roles in the composting process, and their succession may reflect the status of maturing compost (Herrmann and Shann 1997; Ryckeboer et al. 2003). Phospholipid fatty acid (PLFA) analysis is widely used to quantitatively assess the microbial community composition of soils. One of the advantages of this approach is that it covers a wide range of microbial groups, including both bacteria and fungi, in one analysis (Frostegård et al. 2011). However, it has been suggested that nematodes have advantages over soil microbes in terms of their role as biological indicators (Neher 2001). First, by being at a higher trophic level, their assemblages integrate the physical, chemical, and biological properties related to their food resources. Second, their generation times (days to months) are longer than those of metabolically active microbes (hours to days), rendering their assemblages less responsive to short-term fluctuations, such as those resulting from ephemeral nutrient flushes (Nannipieri et al. 1990).

These nematode-specific advantages prompted us to explore the potential of nematodes in assessing the maturity and quality of compost (Chapter 2 and 7). A shift in species composition was observed during the composting process (Chapter 2). Mature composts appear to harbor a typical assemblage of bacterivorous, fungivorous, and bacterivorous/predatory nematodes. The changing trophic structure, expressed as the relative contributions of bacterivorous versus fungivorous nematodes (f/b ratio), proved to be an especially promising proxy for compost maturity (Chapter 2). However, the underlying assumption that nematodes reflect changes in the microbial community has not yet been tested. So far, this has limited our ability to relate nematode community patterns to microbial patterns. The available evidence that changes in the occurrence and abundance of various trophic groups of nematodes are associated with changes in the food web, is mostly limited to soils or aquatic systems. For example, increasing microbial activity associated with the decomposition of barley roots was rapidly followed by an increase in

the proportion of gravid female bacterial-feeding nematodes and then by an increase in total populations (Griffiths and Caul 1993). Another example of bottom-up effects resulted from experiments showing that poultry, but not farmyard manure, increased the relative abundance of bacterial-feeding species (Griffiths et al. 1994). In contrast, De Mesel et al. (2004) demonstrated the significant top-down effect of bacterial-feeding nematodes on the composition of a bacterial community, even at low grazer densities. This effect of the nematodes on the diversity of the microbial community was, however, only observed under high grazing pressure from *Panagrolaimus paetzoldi*. In the early stages of organic matter decomposition, at C/N ratios ≥ 30, fungal abundance, as derived from PLFAs, was lower in the presence of the fungivorous nematode *Aphelenchoides composticola* than in its absence (Chen et al. 2001).

In terrestrial stand-alone decomposition processes, such as composting, the link between the microbial community and metazoan organisms remains especially poorly understood. The objectives of this study were to: (1) analyze the generality of nematode community succession patterns during the composting process and the clear shifts that occur between the composting phases as described in chapter 2, (2) assess whether the profiles and dynamics of the microbial and nematode communities follow similar patterns and whether shifts in one community can be related to shifts in the other, and (3) compare the potential of the nematode community vs. that of the microbial community as potential indicators of compost maturity.

4.3. MATERIALS AND METHODS

4.3.1 Study site and sampling

The compost was prepared according to the Controlled Microbial Composting (CMC) method ("Leubke compost") (Diver 2004). The composting is carried out in windrows and during the composting process, parameters like CO_2 and temperature are intensively monitored. The compost is aerated by turning the compost heap and can be covered to prevent nutrient leaching and/or reduce gaseous emissions and heat loss. Often, a microbial starter is added to inoculate the compost (Diver 2004), but this was not the case in the examined compost. The examined compost heap was located at the Institute for Agricultural and Fisheries Research in Merelbeke, Belgium. The heap was 15 m long, 3 m wide and 1.5 m high, comprised 3 m^3 feedstock materials per lineal meter and consisted of 43% (%, vol/vol) fine poplar bark wood chips, 43% grass seed hay and 14% fresh grass with C/N ratios of 90/1, 30/1 and 15/1, respectively. The composting process examined here actually is a repetition of one that was

incubated exactly one year earlier, and is composed of the same feedstock materials, in the same proportions and at the same composting site (Chapter 2).

On each of 20 consecutive sampling events, covering the entire 6 months of the composting process (September 2008 to March 2009), 3 composite samples were taken. In addition to a time 0 sample, samples were taken twice a week (12 sampling events) during the first six weeks; once a week in the next two weeks (2 sampling events); and once every two weeks for the next 4 weeks (2 sampling events). Subsequently, during the second 3-month period of the process, the heap was sampled once a month (3 sampling events). Hence, samples were taken at days 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 49, 56, 70, 84, 112, 140 and 162 after the start of the composting process.

Each composite sample consisted of 20, randomly picked and thoroughly mixed compost samples of 50 ml. Of this total volume of 1l, a subsample of 400 ml was taken for nematode extraction. For a mathematical justification of taking a subsample from a bulk sample to estimate nematode population densities, see (Been and Schomaker 2006). The remainders of the composite samples (± 600 ml) were freeze-dried (Christ, Gamma 1-20, Osterode am Harz, Germany), ground, and stored at -20 °C for carbon, nitrogen and PLFA analyses.

4.3.2 Abiotic data

Temperature was monitored every hour during the composting process at a depth of at least 30 cm using 5 data-loggers (Testo 175-T2, Ternat, Belgium). Ambient temperature data were obtained from the Royal Meteorological Institute (RMI) weather station, located within 3 km from the sampling site. At every sampling time, moisture content, pH and C/N ratio were measured using material from the composite samples. 20 g compost was shaken by hand in 100 ml distilled water, 3 times with 2-h intervals, and the pH was measured with standard electrodes (Consort P400, Turnhout, Belgium). Moisture content was calculated by determining the dry weight (DW) of 50 ml compost after drying for 48 h at 102 °C. Total C- and N-content was measured with a Variomax CNS element analyzer on 1 g of freeze-dried (Elementar GmbH, Hanau, Germany) compost applying the Dumas method.

4.3.3 Nematode community

Nematodes of each sample (400 ml) were extracted using the modified Baermann funnel method (tray 49 x 37.5 cm, basket 38.5 x 19.5 cm) (Hooper 1986), counted, and 100 individuals were randomly picked using a stereomicroscope (Leica Mz95). For light microscopy observations the specimens were collected in a very small drop of water in an embryo dish. Formaldehyde (4% with 1% glycerol) was heated to 70 °C and an excess (4-5 ml) was quickly added to kill and

preserve the nematodes (Seinhorst 1966). The preserved nematodes were processed to anhydrous glycerol following the glycerol-ethanol method (Seinhorst 1959; as modified by De Grisse 1969) and mounted on aluminium slides with double cover slips (Cobb 1917), or on mass slides (slide 40 x 76 mm; cover glass 34 x 60 mm). Nematodes were identified to genus or, if possible, to species level. The abundance of every genus or species in each sample was expressed as individuals/100 gram dry weight (DW) compost using relative abundance and total count. The nematode genera were assigned to the "coloniser-persister" cp-scale according to their r and K life history characteristics (Bongers 1990) and also classified according to their feeding type (Yeates et al. 1993, see also 1.4.4). These allocations were used to calculate the Maturity Index (MI) (Bongers 1990) and the fungivores/bacterivores ratio (F/F+B ratio) (Yeates et al. 1993), respectively. In the F/F+B ratio, only the fungal and bacterial feeders *sensu stricto* were included, i.e. bacterial-feeding/predators such as some diplogasterid nematodes were not included because their feeding habit can be either bacterivorous, predatory (see Chapter 3) or even fungivorous; furthermore their behavior most likely changes during the compost process. Nematode diversity during the process was expressed using the Shannon-Wiener index, which is mainly determined by evenness among taxa and the Margalef index, which measures taxon richness (Magurran 2004).

4.3.4 Microbial community

Phospholipid fatty acids (PLFAs) of samples of all sampling days except for days 24, 31, 38, 49, 70 and 112 were extracted using a modified Bligh and Dyer (Bligh and Dyer 1959) technique (Moeskops et al. 2010). For the unsaturated fatty acids, the shorthands refer to the number of carbon atoms, the number of double bond(s), and their position as counted from the methyl group end (ω). The suffix c (cis) refers to isomers. The shorthand notation for saturated fatty acids is the same, except for the location of the cyclopropyl (*cy*) ring and the presence of a methyl group (*Me*), either on iso (*i*) or anteiso (*a*) carbon. Briefly, lipids were extracted from 1.00 g freeze-dried compost with a one-phase mix of phosphate buffer (pH 7.0), chloroform and methanol in a 0.9:1:2 ratio (vol:vol). Lipids were then fractionated into neutral lipids, glyco- and phospholipids using SiOH SPE cartridges (Chromabond, Macherey-Nagel GmbH, Düren, Germany). Neutral lipids and glycolipids were discarded and the phospholipid fraction was subjected to mild alkaline methanolysis to give fatty acid methyl esters (FAMEs). These were dried under N_2 atmosphere and re-dissolved in 300 µl hexane containing 8 ppm 19:0 nonadecanoic acid methyl ester (Sigma-Aldrich Inc., St. Louis, USA) as an internal standard. Samples were analyzed by capillary gas chromatography–mass spectrometry (Thermo Focus GC coupled to Thermo DSQ MS; Thermo Fischer Scientific Inc., Waltham, USA), using splitless

injection, helium as a carrier gas and a Restek Rt-2560 capillary column (100 m length x 0.25 mm internal diameter, 0.2 µm film thickness; Restek, Bellefonte, USA). FAMEs were identified by comparison with the chromatographic retention times of a standard quantitative FAME mixture (Restek Food Industry FAME mix 35077) and a qualitative FAME mixture (Supelco Bacterial Acid Methyl Ester mix 28039U, to which individual standards of *a*16:0, *a*17:0, 16:1ω5, *10Me*16:0 and *10Me*18:0 were added). FAMEs were quantified using serial dilutions of the quantitative FAME mix. For FAMEs for which only qualitative standards were available, the calibration curve of the nearest analogous component was used, so that the concentration of all FAMEs could be expressed in nmol g^{-1} dry soil. Only FAMEs which could be accurately identified based on the retention time of known standards were measured and included in further analysis; no major peaks remained unidentified in any of the sample chromatograms. Out of a total of 37 identified FAMEs , only those that were present in proportions of more than 1% of the total, were retained for further analysis and summed to give 'total PLFA' (n= 24). Taxon-specific markers were as in (Moeskops et al. 2010), except that *i*14:0 was used as an additional marker for Gram-positive bacteria (Zelles 1999), *cy*19:0 as an additional marker for Gram-negative bacteria (Zelles 1999) and 18:1ω9c as an additional fungal biomarker (Joergensen and Wichern 2008). The concentration of each marker fatty acid was scaled with the organic C present in each sample. The total fungal community was represented by the sum of 18:1ω9c and 18:2ω6,9 while the sum of 10*Me*16:0 and 10*Me*18:0 was regarded as an indicator for Actinomycetes. The total bacterial community was assumed to be represented by the sum of the marker PLFAs for Gram-negative (16:1ω7c, 18:1ω7c, *cy*17:0 and *cy*19:0), and Gram-positive (*i*14:0, *i*15:0, *a*15:0, *i*16:0, *a*16:0, *i*17:0 and *a*17:0), and the PLFAs 15:0 + 17:0. The fungi/bacteria ratios were calculated by dividing the respective sums of marker fatty acids. The PLFA 16:1ω5 was found in our study in every sample with the highest concentrations during maturation and has been reported as biomarker of arbuscular mycorrhizal fungi (AMF) (Olsson et al. 1995). However, this marker also occurs in Gram-negative bacteria (Zelles 1999), is a poor indicator of AMF if bacterial biomass is high (Frostegård et al. 2011), and AMF were never reported from compost before. Therefore, this PLFA marker was provided for completeness (Table 4.4) but not included in further data analyses.

4.3.5 Statistical analysis

First, a correlation matrix was calculated (Pearson product moment correlation coefficients (r) in Statistica 6.0 (Statsoft Inc.)) to unravel possible colinearity among abiotic variables (temperature, pH, moisture, C/N ratio, ambient temperature). Subsequently, changes in the abiotic factors during the composting process were analyzed using Principal Component Analysis

(PCA) in Primer 6 (Clarke and Warwick 2001). Total nematode numbers, and the diversity indices across sampling times were compared using one-way ANOVA and subsequent post hoc Tukey HSD tests in Statistica 6.0. For the MI, F/F+B ratio (nematode data), and the total and functional group (Gram-positive, Gram-negative, Actinomycetes, total bacteria, fungi and AMF) PLFA amounts of the different composting phases, assumptions of normality and homogeneity of variances were not fulfilled. Therefore, these data were analysed using non-parametric Kruskall-Wallis and Mann-Whitney U pairwise tests with Bonferroni corrections ($\alpha = 0.016$), in Statistica 6.0. Nematode community compositions (species abundance data) of the different moments during the composting process were compared using non-metric MultiDimensional Scaling (nMDS) and significant differences between composting phases were evaluated using Analysis of Similarities (ANOSIM) in Primer 6. Composting phases were predefined based mainly on the temperature profile of the composting process (see 4.4.1 and Chapter 2). For both nMDS and ANOSIM, Bray-Curtis similarity matrices on square-root transformed abundance data (individuals/100 g DW compost) were used. The relative PLFA concentrations (mol%) of the fatty acid markers were subjected to a PCA, and significant differences between composting phases were investigated using Analysis of Similarities (ANOSIM) in Primer 6. Finally, correlations of the nematode community with the microbial community and vice versa were analyzed. To provide a measure for statistical significance in terms of the canonical relationships among the two sets of variables, a canonical correlation analysis (CANCORR) was done in SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Only the best explaining variables of each of the two sets of variables (nematode and PLFA data) were used, which were selected using the BEST (BIOENV) procedure in Primer 6.

4.4. RESULTS

4.4.1 Abiotic variables

Based on temperature, the composting process is typically divided into three phases: the thermophilic phase from day 1 until day 28, the cooling phase from day 28 until day 84, and the maturation phase from day 84 until day 162. Here, the thermophilic phase showed two distinct heat peaks: The first was from day 3 to day 7 (37 °C on average, with maxima up to 65 °C), and the second was from day 17 to day 23 (38 °C on average, with maxima up to 60 °C). After day 28, the temperature gradually decreased (cooling phase) until it reached 8 to 10 °C (Figure 4.1A), which approximates the ambient temperature (Figure 4.1B). The moisture content fluctuated during the process, with a maximum of 36 ± 2.1% on day 112 (Figure 4.1B). The C/N ratio gradually decreased from 57 ± 3.8 on day 0, to 29 ± 3.8 on day 162 (Figure 4.1C). The pH

increased sharply from 4.8 ± 0.3 on day 0, to 8.1 ± 0.2 on day 10. On day 17, it decreased to 6.7 ± 0.04, and afterwards, it remained more-or-less constant (between 6.9 ± 0.3 and 7.6 ± 0.2) (Fig 4.1C). As expected, the C/N ratio significantly correlated with decreasing compost temperature and increasing pH (Table 4.1). The PCA grouped samples according to composting phase (Figure 4.2). The first two axes of the PCA (Figure 4.2, vector loadings of eigenvectors in Table 4.1) explained 73% of the variation in the abiotic variables. The first axis accounts for 51% of the variation and separates the samples of the various composting phases based on C/N ratio and pH. Along the second axis, which explains an additional 22% of the variation, temperature was the most determining variable.

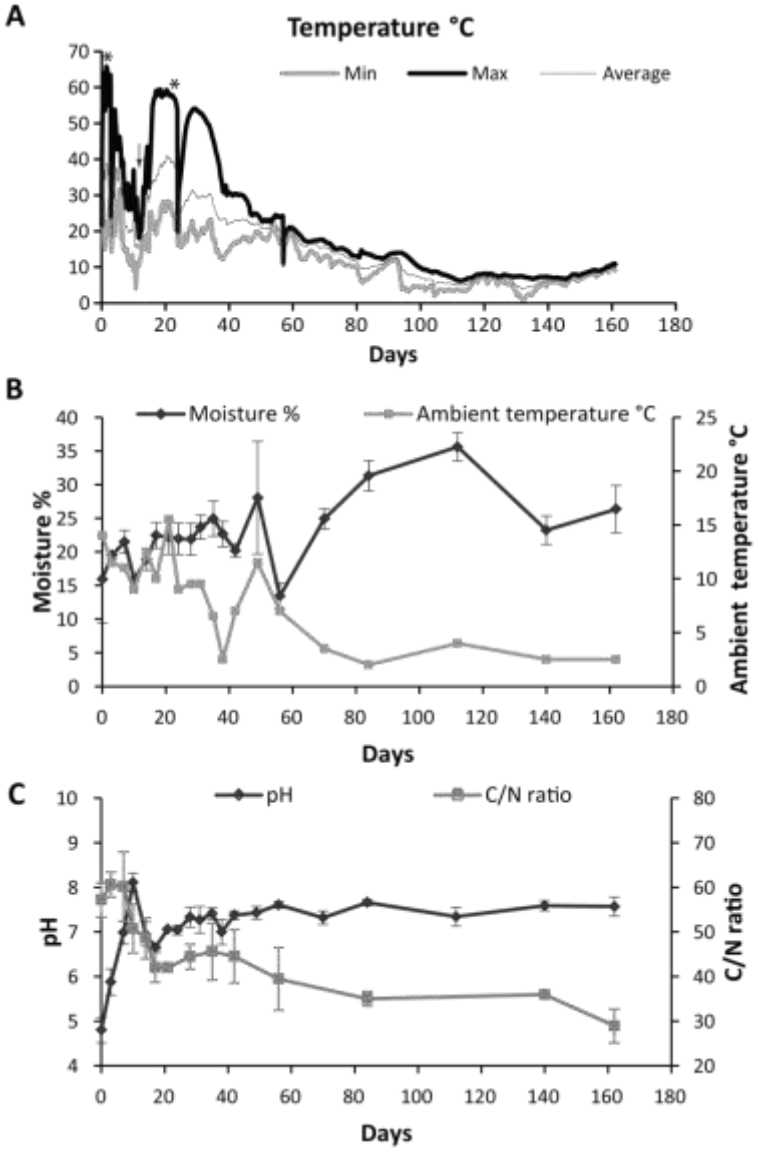


Figure 4.1

Abiotic variables measured during the composting process including (A) Temperature of the compost (°C). Data of 5 dataloggers, programmed to measure temperature every hour, were averaged (Average) and minimum (Min) and maximum (Max) temperatures are displayed. The asterisks indicate the addition of water followed by turning of the heap on day 3 and 24, while the arrow indicates turning of the heap on day 17. (B) Moisture content (%) and mean ambient temperature per day (24 h)(°C). (C) pH values and C/N ratio. Error bars indicate SD (based on 3 replicates).

Table 4.1

Pearson correlation coefficients and product moment correlation coefficients among continuous environmental variables (Temperature, Moisture content, pH, Env. Temperature and C/N ratio) measured during the composting process and vector loadings of the Principal Component Analyses (PCA).

						Vector loadings	
	Temperature	pH	Moisture	C/N	Env. Temp	PCA 1	PCA 2
Temperature	1					-0.42	0.8
pH	-0.26	1				0.48	0.39
Moisture	-0.2	0.28	1			0.43	0.43
C/N	0.71**	-0.53	-0.47	1		-0.63	0.06
Env. Temp	0.78***	-0.61*	-0.53*	0.69**	1	-	-

*p≤0.05; **p<0.01; ***p≤0.001

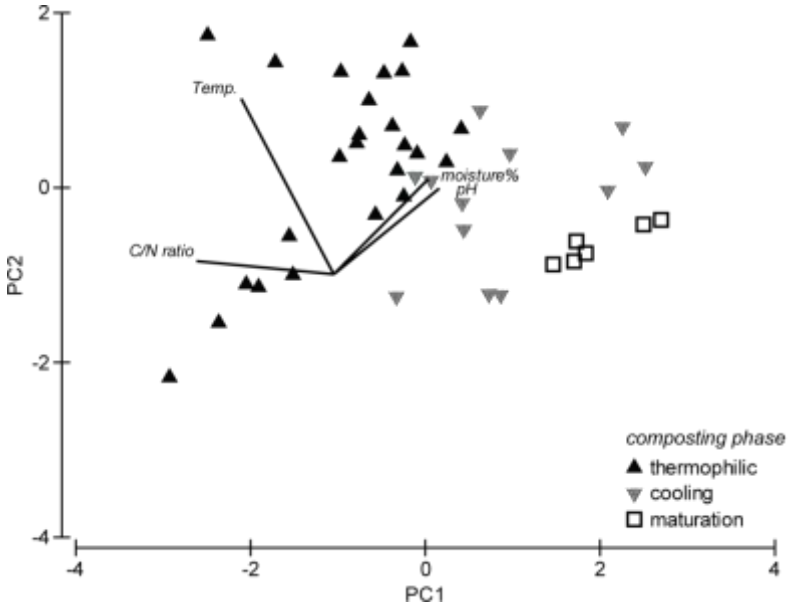


Figure 4.2

Two-dimensional PCA ordination of the abiotic variables of the composting process. The variables used in the analyses are displayed (i.e. temperature, CO_2, pH, moisture content (%), C/N ratio). PC1 (y-axis) and PC2 (x-axis) together account for 67% of the total variation.

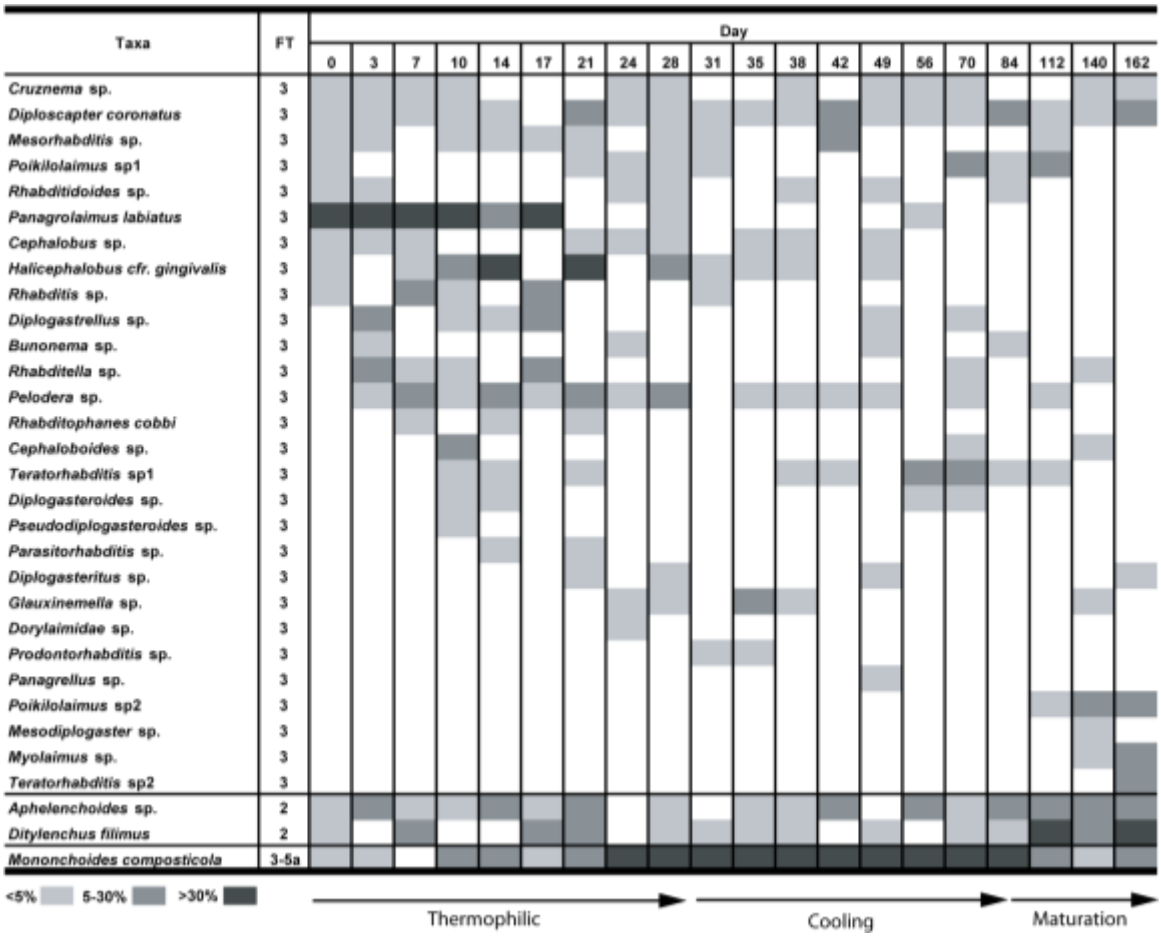
4.4.2 Nematode population dynamics

All samples contained high densities of nematodes (on average 1097 ± 309/100 g DW compost). During the thermophilic phase, nematode numbers were relatively low during the two heat peaks (on average 683 ± 113/100 g DW compost). In between these peaks, however, nematode numbers spectacularly increased up to 3657 ± 1400 nematodes/100 g DW compost. This was concomitant with a temporary temperature drop to 32 ± 5 °C from day 10 to day 14 (Figure 4.3A). The total nematode numbers fluctuated during the cooling phase and remained more-or-less constant (i.e., on average 1046 ± 436 nematodes/100 g DW compost) during maturation. In total, 30 genera that belonged to 14 families were identified (Table 4.2). Irrespective of the index used, nematode diversity remained rather stable (Table 4.3); between four (day 42) and eight

(day 21) genera were recorded at each individual sampling moment. The most common taxa that occurred throughout the composting process were *Aphelenchoides* sp., *Diploscapter coronatus*, *Ditylenchus* sp., *Mononchoides composticola,* and *Pelodera* sp. Other species, such as *Glauxinemella* sp., *Myolaimus* sp., *Panagrolaimus* sp., *Poikilolaimus* sp. 2, and *Teratrorhabditis* sp. 2, were restricted to specific moments in the composting process (Table 4.2). The nMDS visualizes (Figure 4.3B) groupings of nematode communities that broadly correspond with the composting phases; the average nematode species composition was significantly different ($P \leq 0.002$) in each of these predefined phases (ANOSIM). The species composition in the compost was best explained by a combination of the temperature and C/N ratio of the compost heap ($r = 0.49$, stepwise BIOENV). Bacterial-feeding nematodes were present in all compost samples, though most samples contained fungal feeders and predators (days 0, 10, 14 up to 162) as well. From the start of the process up to day 112, the bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogasteridae) were the most abundant, followed by the bacterial-feeding general opportunists (cp-2) (Cephalobidae), and the fungal-feeding general opportunists (cp-2) (Aphelenchoididae). However, clear successional shifts can be distinguished in every composting phase. First, during the thermophilic phase, *Panagrolaimus labiatus* and/or *Halicephalobus* cfr. *gingivalis* (both Panagrolaimidae) were abundantly present (days 0, 3, 7, 10, 14, and 17, and days 14 and 21, respectively). Second, from day 10 onwards, the relative abundances of the bacterial-feeding/predator diplogasterids (mainly *Mononchoides composticola*) gradually increased to > 30% during the cooling phase (from day 28 to 84). Third, during maturation (from day 84 onwards), the relative abundances of the fungal-feeding nematodes increased, specifically the fungal-feeding Anguinidae (mainly *Ditylenchus filimus*) (Table 4.2). The MI and the F/F+B ratio showed a significant increase during the maturation phase as compared to both cooling and thermophilic phases (Table 4.3).

Table 4.2

Nematode species list with indication of relative abundance of the species during the composting process. The feeding type (FT) of each species is also provided (3= bacterial-feeding, 2= fungal-feeding, 3-5a= bacterial-feeding/predator).



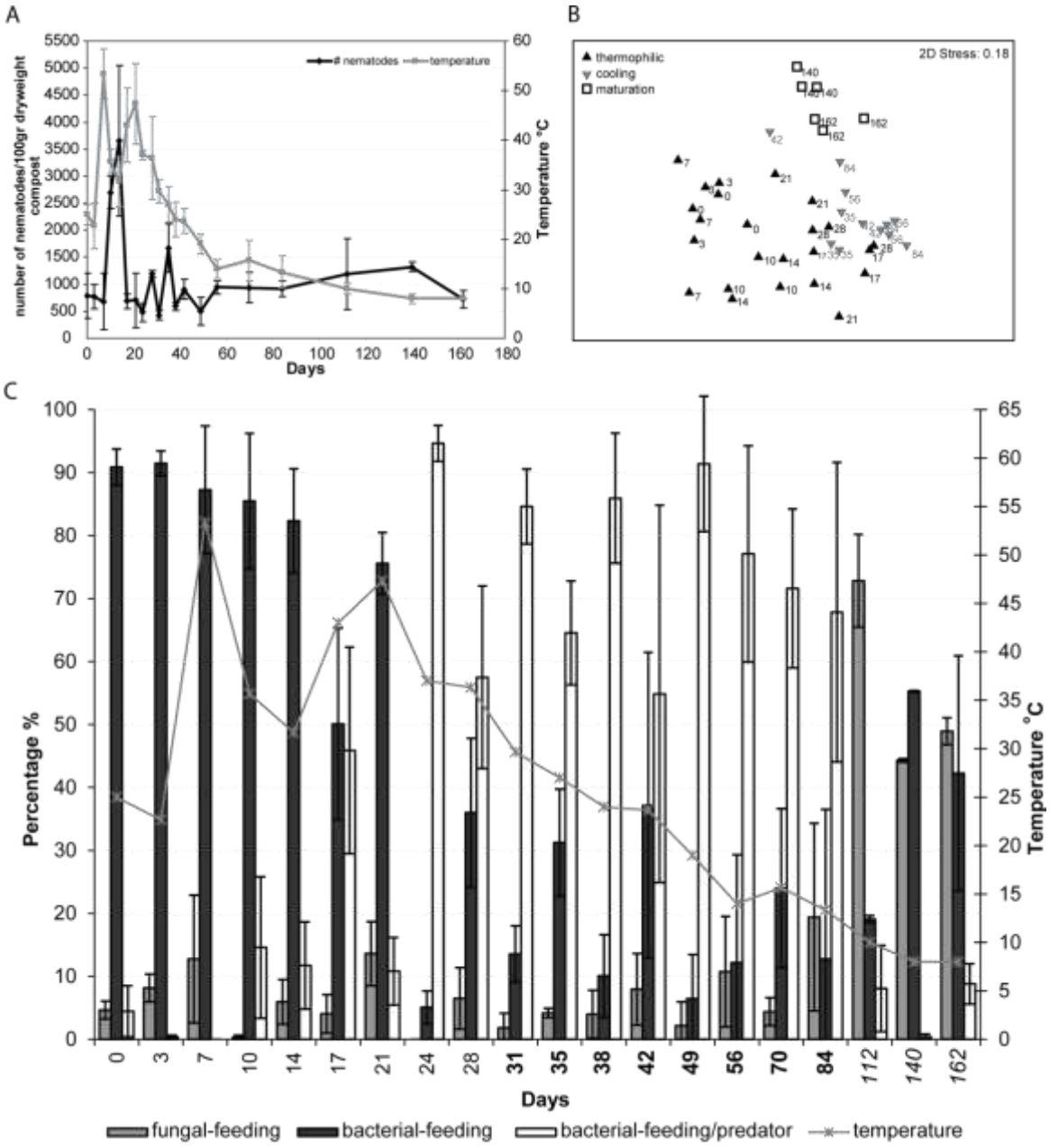


Figure 4.3

(A) Total nematode numbers per 100 g dry weight compost and the temperature of the compost during the process. (B) MDS ordination of square root transformed nematode species abundances of every sampling moment. Time of composting is indicated as a number next to the mark of the sample. (C) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator) at every sampling moment. Omnivores are not represented in the graph because they only occurred in one sample. Samples of the thermophilic, cooling and maturation phases are represented by labels in regular, **bold** and *italic*s, respectively.

Table 4.3

Overview of the means ± SD of the abundance of nematodes (per 100g dry weight compost), number of genera, Shannon-Wiener index (H'), Margalef's diversity index (d), percent conbribution to the functional groups (trophic groups: type 2 (fungal-feeding nematodes), type 3 (bacterial-feeding nematodes), type 3-5a (bacterial-feeding/predator nematodes), type 8 (omnivorous nematodes) and cp-groups: cp-1 (enrichement opportunists), cp-2 (general opportunists), cp-4 (persisters) and the values of the indices: F/F+B (fungivorous/bacterivorous ratio) and MI (Maturity Index) at everey sampling moment based on 3 replicates.

	Day																			
	0	**3**	**7**	**10**	**14**	**17**	**21**	**24**	**28**	**31**	**35**	**38**	**42**	**49**	**56**	**70**	**84**	**112**	**140**	**162**
Abundance	791±416	772±222	672±522	2700±295	3657±1401	683±141	703±506	473±164	1183±61	425±84	1667±449	586±69	905±187	498±251	881±102	938±286	912±143	1185±663	1323±82	722±158
# Genera	7±1	6±2	5±4	7±3	7±5	6±2	8±1	4±2	7±3	5±2	7±2	5±3	5±1	7±5	5±4	6±1	4±4	5±1	7±1	7±1
H'	0.9±0.18	1.2±0.35	1±0.34	1.5±0.37	1.3±0.43	1.1±0.62	1.4±0.36	0.3±0.23	1.2±0.61	0.7±0.4	1.1±0.06	0.5±0.65	0.7±0.42	0.6±0.7	0.8±0.6	0.8±0.44	0.7±084	1.2±0.1	1.5±0.24	1.6±0.18
d	1±0.02	0.8±0.27	0.8±0.96	0.9±0.44	0.8±0.54	0.7±0.3	1.2±0.06	0.6±0.36	0.9±0.45	0.7±0.15	0.9±0.21	0.7±0.59	0.5±0.07	1±0.89	0.6±0.42	0.8±0.11	0.5±0.59	0.6±0.18	0.9±0.08	1±0.1
	Functional groups %																			
Trophic groups %																				
type 2	4.7±2.5	8.15±3.8	12.75±17.6	0.29±0.6	5.94±6.1	4.05±5.3	13?61±8.8	0	6.51±8.5	1.8±4	4.2±1.3	3.99±6.6	7.94±9.9	2.16±6.6	10.7±15.2	4.39±3.9	19.41±25.8	72.82±12.8	44.3±0.5	48.93±37
type 3	90.9±5	91.49±3.4	87.25±17.6	85.48± 8.6	82.34±14.3	50.07±26.3	75.58±8.5	5.07±4.5	35.99±20.5	13.56±7.7	31.25±14.7	10.07±11.3	37.2±42	6.45±12.1	12.1±14.5	24.02±18.6	12.75±15.4	19.1±1	55.27±0.2	42.26±32.3
type 3-5a	4.5±7	0.36±0.5	0	14.6±19.5	11.72±12	45.88±28.4	10.8±9.3	94.64±4.9	57.5±25.1	84.62±10.3	64.55±14.3	85.94±17.9	54.86±51.9	91.38±18.6	77.1±29.7	71.59±21.9	67.84±41.2	8.08±11.8	0.42±0.7	8.81±5.8
type 8	0	0	0	0	0	0	0	0.29±0.6	0	0	0	0	0	0	0	0	0	0	0	0
Cp-groups %																				
cp-1	93.75±6.1	88.97±8.6	87.19±17.4	99.71±0.6	94.06±6.1	95.58±5	84.79±10.6	99.2±1	93.15±8.3	98.19±4	93.81±3.7	95.3±7.1	92.06±9.9	97.69±7.2	58.92±15.2	95.61±3.9	80.59±25.8	27.18±12.18	55.69±0.5	51.07±37
cp-2	6.25±6.1	11.03±8.6	12.81±17.4	0.29±0.6	5.94±6.1	4.42±5	15.21±10.6	0.51±1.1	6.85±8.3	1.81±4.04	6.18±3.7	4.69±7.1	7.94±9.9	2.3±7.2	0±15.2	41±3.9	19.41±25.8	72.82±12.8	44.31±0.5	48.93±37
cp-4	0	0	0	0	0	0	0	0.29±0.6	0	0	0	0	0	0	0	0	0	0	0	0
	Indices																			
F/F+B	0.05±0.02	0.08±0.04	0.13±0.18	0.003±0.01	0.07±0.06	0.07±0.07	0.11±0.1	0	0.11±0.15	0.09±0.16	0.13±0.07	0.11±0.19	0.12±0.11	0.28±0.25	0.24±0.24	0.16±0.17	0.35±0.32	0.78±0.04	0.45±0.001	0.56±0.26
MI	1.08±0.06	1.13±0.09	1.14± 0.17	1.0± 0.0	1.06±0.06	1.04±0.05	1.12±0.11	1.0±0.02	1.07±0.08	1.02±0.04	1.06±0.04	1.05±0.07	1.1±0.1	1.05±0.07	1.11±0.15	1.04± 0.04	1.17± 0.26	1.71± 0.12	1.44±0	1.45± 0.37

4.4.3. Microbial community dynamics

The total PLFA concentration (Table 4.4) showed no significant differences between the compost phases. It was lowest during the first heat peak, on day 3 (465 ± 60 nmol/g), and after the second heat peak, on day 35 (518 ± 103 nmol/g). During the cooling and maturation phases, however, total PLFA fluctuated between 528 ± 110 and 776 ± 93 nmol/g (Table 4.4). On average, 67% of the total PLFA (min = 35%, max = 74%) could be used as biomarkers for a functional microbial group. The PCA of the relative PLFA concentrations of the biomarkers resulted in a clear discrimination, mainly along the first axis, between compost of the early thermophilic phase (days 0, 3, 7, 10, 14, and 17) and compost of the cooling and maturation phases (days 35, 42, 56, 84, 140, and 162) (Figure 4.4A). The first axis explained 39% of the variation, and the second explained an additional 31%. The marker fatty acids of bacteria (increasing 16:1ω7c and 18:1ω7c and decreasing *i*17:0 and 10*Me*16:0) contributed the most to the first axis, while the fungal marker 18:2ω6,9c contributed the most to the second axis (Figure 4.4B). The average marker fatty acids compositions (mol%) were significantly different in the the thermophilic phase than they were in the cooling phase (ANOSIM). In the first sample, before the temperature began to increase, fungi and bacteria contributed equally to the microbial community. With increasing temperature during the first heat peak (days 3-7), the amount of Gram-negative and Gram-positive bacteria and Actinomycetes PLFAs began to increase, while the fungal biomass decreased (Figure 4.4C). During the intermediate temperature drop (days 10-17), the Gram-negative bacteria reached their maximum and the fungi began increase again, while the Gram-positive bacteria continued to increase and reached their maximum levels during the second heat peak (days 17-28). During the early cooling phase, both the fungi and the Actinomycetes reached their maximum concentrations; the fungi concentration was relatively stable during the remainder of the compost process, being significantly higher than during the thermophilic phase (Table 4.4). The Actinomycetes' concentration, however, slightly decreased again. During maturation, the Gram-negative bacteria were significantly more abundant than during cooling (Table 4.4). The fungi/bacteria ratio (based on PLFA) (Figure 4.5) was low (< 1) during the thermophilic phase, but it increased significantly ($P < 0.001$) and remained relatively constant (± 1) during the subsequent cooling and maturation phases. This increase in the fungi/bacteria ratio did not coincide with an increase in the nematode-based equivalent (F/F+B), which only began to increase at the onset of the maturation phase.

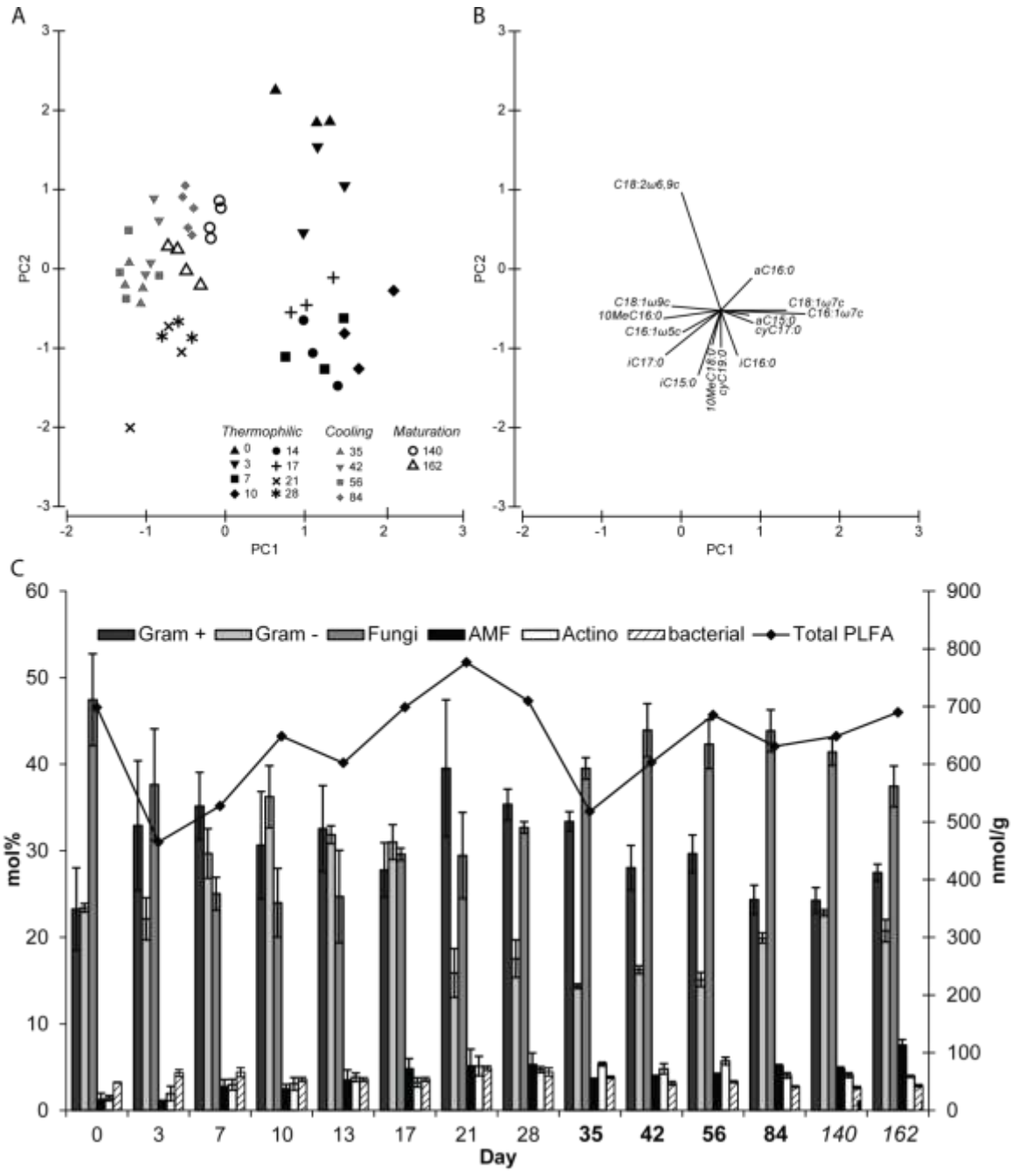


Figure 4.4

(A) Two-dimensional PCA ordination of biomarker fatty acids (mol%). (B) Vector loading plot with all individual marker fatty acids. (C) Percent contributions of functional groups (mol%) (bars) and total PLFA concentration (nmol/g) (line) during the composting process.

Table 4.4

Total PLFA and main biomarker concentrations (nmol g^{-1}dry soil) ± standard deviation, during the composting process. Different letters after means indicate significant differences between composting phases using pairwise Mann-Whitney U tests with Bonferroni corrections (α = 0.016). If no letters no significant differences were found.

Day & Phase		*total PLFA*	*Gram +*	*Gram -*		*Actino-mycetes*		*Total Bacteria*	*Fungi*		*AMF**	
Thermophilic	***0***	698±307	57.4±7.18	60.6±20.46		3.8±2.08		126.4±30.31	126.1±57.46		2.8±0.96	
	3	465±60	70.3±17.36	47.1±3.89		4.1±1.56		126.7±14.26	80.4±14.71		2.3±0.33	
	7	528±110	108.2±17.9	93.2±26.76		9.1±2.27		214.9±42.42	78.8±22.36		8.7±4	
	10	648±240	119.2±18.57	149.8±61.36	*ab*	12±2.6	*a*	283.3±80.89	101±46.18	*a*	10.5±4.9	*a*
	13	602±259	125.5±40.57	126.5±49.57		14.8±4.55		266.2±95.95	103.6±53.91		15.2±9.05	
	17	699±29	131±18.57	145.9±8.31		15.3±3.02		293.7±16.79	139.4±4.86		22.6±5.58	
	21	776±94	196±21.69	80.4±20.24		25.5±3.5		300.8±8.43	149±35.27		26.3±11.4	
	28	710±118	165.1±20.15	81.6±11.02		21.9±1.75		267.5±27.43	152.5±15.31		25.2±8.63	
Cooling	***35***	518±103	122.9±26.12	52.6±10.2		19.6±3.41		189.5±33.56	145.1±26.41		13.2±2.52	
	42	603±51	126.5±20.98	73.1±7.51	*a*	21.7±4.14	*b*	213.7±25.9	196.4±7.10	*b*	17.3±2.07	*a*
	56	685±152	152±37.52	77.1±17.26		29.3±7.73		245.9±49.94	215.4±43.43		22.4±3.04	
	84	631±82	116.6±16.02	95.6±13.86		19.3±181		225.3±30.91	210.1±29.35		24.5±3.52	
Maturation	***140***	648±50	121±13.65	113.7±7.72	*b*	20.3±2.49	*ab*	247.9±18.73	206±12.58	*b*	23.8±2.48	*b*
	162	690±40	141.4±10.17	107±11.47		20.3±0.85		263±18.78	192.5±13.77		38.9±4.88	

* The used biomarker for AMF is questionable in a compost environment (see materials and method section).For completeness the concentrations of this group are provided but not used in further community analyses.

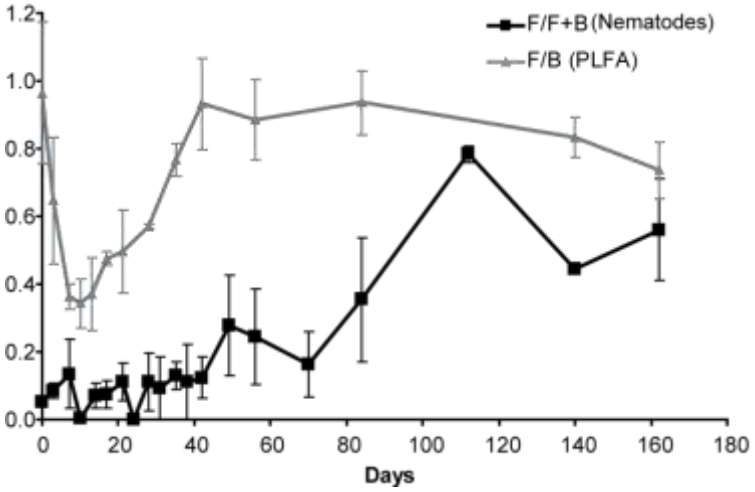


Figure 4.5

F/B ratio based on fungal and bacterial PLFA data and F/F+B ratio based on the fungal-and bacterial-feeding nematode densities during the composting process. Data are means ± SD of 3 replicates indicated as error bars.

4.4.4. Relationships between microbial and nematode communities

The BEST procedure indicated the fatty acids 10*Me*16:0, 10*Me*18:0 (both indicators of Actinomycetes), and 18:2ω6,9 (an indicator of fungi) as the variables best explaining the microbial community pattern during the composting process (r = 0.43) and the nematode taxa *Glauxinemella* sp., *Mononchoides composticola*, *Myolaimus* sp., *Rhabditella* sp., and *Teratorhabditis* sp1 as best explaining shifts in the nematode community structure during the composting process (r = 0.39). The canonical correlation analyses between these two sets of selected variables revealed a significant (P = 0.036) first axis canonical correlation (r = 0.64). *Mononchoides composticola* and *Rhabditella* sp. contributed most to the nematode-based first canonical variable, while 10*Me*18:0 and 18:2ω6,9 contributed most to the PLFA-based canonical variable.

4.5. DISCUSSION

The nematode assemblage and succession observed here are remarkably similar to those of an earlier study in 2006-2007 (Chapter 2). To the best of our knowledge, this was the first study on nematode community dynamics during a composting process. Although, in this former study, nematodes were absent during the thermophilic phase, the nematode abundances of the final composting stages were similar. The presence of nematodes during the heat peak in the present study may be explained by the strong environmental temperature drop during the thermophilic phase. This may have resulted in the patchiness of high-temperature spots and thus the presence of refuges where nematodes could escape the most extreme temperatures. Most importantly, the shifts between the functional groups showed the same pattern: from a thermophilic phase dominated by bacterial-feeding nematodes – at first exclusively enrichment opportunists and later supplemented with general opportunists – to a community with increasing numbers and the eventual dominance of bacterial-feeding/predator enrichment opportunists (mainly *Mononchoides*) in the cooling phase to the maturation phase, in which the fungal-feeding general opportunists became more important. These functional groups are also largely represented by the same taxa as in the 2006-2007 study (Chapter 2), i.e., *Aphelenchoides* sp., *Diploscapter coronatus,* and *Mononchoides* sp., together with *Pelodera* sp. and *Ditylenchus filimus,* were the most common species. The current study confirms that the nematode functional group shifts correspond to the three composting phases and that the F/F+B ratio and MI significantly increase during maturation. Except for temperature, the BIOENV procedure selected the C/N ratio as variable best explaining nematode community structure. Apparently,

the considerably lower moisture content (< 30%) at most of the sampling points, as compared to the study of 2006-2007 (Chapter 2), did not significantly affect the nematode community. However, it should be noted that these differences in moisture content might also be related to the potentially different organic matter contents of the composts because high levels of organic matter content increase the water holding capacity.

The microbial community during composting has been better studied than the nematode community. In accordance with Steger et al. (2003) and Bolta et al. (2003), the total PLFA, as a measure of total microbial biomass, remained more-or-less stable during the composting process. In contrast, Klamer and Bååth (1998) and Hellmann et al. (1997) described an increase during the thermophilic phase and a subsequent decrease of total PLFA during the remainder of the composting process. The overall succession of the microbial community, as described in this study, has already been reported (Herrmann and Shann 1997; Klamer and Bååth 1998; Steger et al. 2003; Ryckeboer et al. 2003). Fungal biomass is high at the onset of the composting process, after which it sharply declines when temperatures peak. It increases again when temperatures drop below 45°C during cooling and maturation. Gram-positive bacteria dominate during the thermophilic phase, while Gram-negative bacteria increase after the thermophilic phase. This pattern can be explained by the higher sensitivity of fungi to high temperatures and their slower recovery after heating than bacterial communities (Bárcenas-Moreno and Bååth 2009). However, this does not agree with Carpenter-Boggs et al. (1998), who observed no significant changes in fungal biomarkers during composting, and Bolta et al. (2003) found a decrease in fungal biomass with compost time. Most authors (Hellmann et al. 1997; Klamer and Bååth 1998; Steger et al. 2003; Bolta et al. 2003; and in this study) found higher levels of Actinomycetes in the later composting stages, although Herrmann and Shann (1997) reported high levels during the thermophilic phase. Steger et al. (2007) already suggested that Actinomycetes may be a good indicator of compost maturity based on the changes in species composition and their increased share in the microbial communities during the later compost stages. Our BEST analyses also selected the biomarkers of Actinomycetes (i.e., 10*Me*16:0 and 10*Me*18:0) as being among the variables best explaining the composition of the nematode community. The explanations of several more-detailed aspects of microbial community structure in our study also correspond with those of previous studies. For example, the increase of Gram-positive bacteria with increasing temperature during the thermophilic phase corresponds to an increase in iso-fatty acids, while the level of anteiso-fatty acids remained more-or-less constant during the process (Klamer and Bååth 1998). High levels of monounsaturated PLFAs, such as the iso-fatty acids and 16:1ω7 and 18:1ω7, indicate high substrate availability (Moore-Kucera and Dick

2008). This may be especially true during the thermophilic phase, when readily available substances of the feedstock materials are decomposed mainly by thermophilic Gram-positive bacteria when temperatures are high (> 50 °C) and mainly by Gram-negative bacteria when temperatures are low (< 50 °C). The decomposition of complex structural compounds, such as lignin and lignocellulose, is generally assumed to take place during the cooling and the maturation phase because it is mostly performed by fungi (Vargas-Garciá et al. 2012). Indeed, the fungal biomass was low during the first heat peak of the thermophilic phase (on day 7), and from this point onwards, it began to increase from cooling until the beginning of the maturation phase, when it remained more-or-less constant. However, fungal-feeding nematodes did not increase until the beginning of the maturation phase (day 112). This delayed increase of fungal-feeding nematodes is also illustrated by the different timings of the maximum values of the F/B (based on the microbial community) and those of the F/F+B (based on nematodes). There are several possible explanations for the delayed increase of fungal-feeding nematodes. First, the relatively long generation time of cp-2 fungal-feeding nematodes (Ferris et al. 2001) and the high maximum temperatures in the beginning of the cooling phase might have hampered the development of the fungal-feeding nematodes. The inhibition of the reproduction of the fungal-feeding taxa *Aphelenchoides rutgersi* at 33 °C and *Ditylenchus* sp. at 39 °C has been reported based on *in vitro* cultures (Pillai and Taylor 1967; Moens et al. 1996). Secondly, nematode predators also may have contributed to the delayed increase in the number of fungal-feeding nematodes. During the cooling phase, when the microbial community was shifting from bacterial dominance to more-or-less equal shares of fungi and bacteria, the nematode population was dominated by the bacterial-feeding/predator *Mononchoides composticola*, which exploited its nutritional advantage in this changing environment, i.e. being able to feed on both bacteria and on other compost nematodes (Chapter 3, part 2). Furthermore it is possible that they are capable of feeding on fungi as well, as has been demonstrated for some other diplogasterid taxa, including *Pristionchus pacificus* (von Lieven and Sudhaus 2000), making them excellent generalists. Despite this delay, the microbial and nematode communities largely followed the same pattern, as was confirmed by the canonical correlation analysis.

In conclusion, although the microbial and the nematode communities showed largely similar patterns, based on the microbial community, it is only possible to discriminate between the thermophilic phase and the rest of the process. There is bacterial dominance during the thermophilic phase, and there are more-or-less equal shares of bacteria and fungi during cooling and maturation. However, based on the nematode community, it is possible to discriminate between the three composting phases: dominance of bacterial-feeding nematodes during the

thermophilic phase, dominance of bacterial-feeding/predators (diplogasterids) during cooling, and an increased abundance of fungal-feeding nematodes during maturation. Furthermore, the PLFA method has the drawback of being much more expensive, time-consuming, and labor-intensive than the extraction and identification (up to family level or feeding type) of the nematode community. Therefore, this study demonstrates that the nematode community holds promise as a tool for use in assessing compost maturity. However, further research on more compost processes is warranted in order to transform the characteristics of the nematode community into threshold values for mature compost.

Acknowledgements

Hanne Steel acknowledges the Research Foundation - Flanders (FWO-Flanders, Belgium) for providing a PhD grant as aspirant. David Buchan is the recipient of a PhD grant from the Special Research Fund of Ghent University (BOF project 01J16907). We are grateful to Annelies Scholaert and Lien Boshoff for their help with sampling and identification. We want to thank Koen Willekens and Bart Vandecasteele for providing the opportunity to sample the composting processes at the Institute for Agricultural and Fisheries Research in Merelbeke, Belgium.

4.6. REFERENCES

Bailey, K.L., Lazarovits, G., 2003. Suppressing soil-borne diseases with residue management and organic amendments. SoilTill. Res. 72,169-180.

Bárcenas-Moreno, G., Bååth E., 2009. Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. Soil Biol. Biochem 41, 2517-2526.

Been, T.H., Schomaker, C.H., 2006. Distribution patterns and sampling. In: Perry, R.N., Moens, M.(Eds.), Plant Nematology. CABI publishing, Wallingford, pp. 302-326.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem.Phys. 37, 911-917.

Bolta, S.V., Mihelic, R., Lobnik, F., Lestan, D., 2003. Microbial community structure during composting with and without mass inocula. Compost Sci.Util. 11, 6-15.

Bongers, T., 1990. The Maturity Index - An Ecological Measure of Environmental Disturbance Based on Nematode Species Composition. Oecologia 83, 14-19.

Butler, T.A., Sikora, L.J., Steinhilber, P.M., Douglass, L.W., 2001. Compost age and sample storage effects on maturity indicators of biosolids compost. J. Environ. Qual. 30, 2141-2148.

Carpenter-Boggs, L., Kennedy, A.C., Reganold, J.P., 1998. Use of phospholipid fatty acids and carbon source utilization patterns to track microbial community succession in developing compost. App. Environ. Microb. 64, 4062-4064.

Chen, J., Ferris, H., Scow, K.M., Graham, K.J., 2001. Fatty acid composition and dynamics of selected fungal-feeding nematodes and fungi. Comp. Biochem. Phys. B. 130, 135-144.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth.

Cobb, N.A.,. 1917. Notes on nemas. Contribution to a Science of Nematology 5, 117-128.

Cogger CG. 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Science & Utilization 13(4):243-51.

De Grisse, A.T., 1969. Redescription ou modifications de quelques technicques utilisées dans l' étude des nématodes phytoparasitaires. Mededelingen van de faculteit landbouwwetenschappen, Rijksuniversiteit Gent.

De Mesel, I., Derycke, S., Moens, T., Van der Gucht, K., Vincx, M., Swings, J., 2004. Top-down impact of bacterivorous nematodes on the bacterial community structure: a microcosm study. Environ.Microbiol. 6, 733-744.

Diver, S., 2004. Controlled Microbial Composting and Humus Management: Leubke Compost. ATTRA- National Sustainable Agriculture Information Service; Available from.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Frostegård, A., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621-1625.

Griffiths, B.S., Caul, S., 1993. Migration of bacterial-feeding nematodes, but not protozoa, to decomposing grass residues. Biol. Fert.Soils 15, 201-207.

Griffiths, B.S., Ritz, K., Wheatley, R.E., 1994. Nematodes As Indicators of Enhanced Microbiological Activity in A Scottish Organic Farming System. Soil Use Manage. 10, 20-24.

Hellmann, B., Zelles, L., Palojarvi, A., Bai, Q.Y., 1997. Emission of climate-relevant trace gases and succession of microbial communities during open-window composting. Appl.Environ. Microb. 63, 1011-1018.

Herrmann, R.F., Shann, J.F., 1997. Microbial community changes during the composting of municipal solid waste. Microb. Ecol. 33, 78-85.

Hooper, D.J., 1986. Extraction of free-living stages from soil. In: Southey, J.F. (Ed.),, Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 5-30.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977-2991.

Klamer, M., Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. Fems Microbiol. Ecol. 27, 9-20.

Kutzner, H.J. 2000. Microbiology of composting. In: Rehm, H.J., Reed, G., (Eds.),Biotechnology. Wiley-VCH, pp. 35-100.

Magurran, A.E., 2004. Measuring biolological biodiversity, Blackwell Science Ltd., Oxford, UK, 215 pp.

Moens, T., Coomans, A., DeWaele, D., 1996. Effect of temperature on the in vitro reproduction of Aphelenchoides rutgersi. Fund.App. Nematol. 19, 47-52.

Moeskops, B., Sukristiyonubowo, Buchan, D., Sleutel, S., Herawaty, L., Husen, E., Saraswati, R., Setyorini, D., De Neve, S., 2010. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. Appl. Soil Ecol. 45, 112-120.

Moore-Kucera, J., Dick, R.P., 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb. Ecol. 55, 500-511.

Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological activity in soil. In: Bollag, J.M., Stotzky, G. (Eds.), Soil Biochemistry. Marcel Dekker, New York, pp. 293-355.

Neher, D.A., 2001. Role of Nematodes in Soil Health and their Use as Indicators. Journal of Nematology 33, 161-168.

Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments--A review. Applied Soil Ecology 44, 101-115.

Olsson, P.A., Baath, E., Jakobsen, I., Soderstrom, B., 1995. The use of phospholipid and neutral lipid fatty-acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol. Res. 99, 623-629.

Pillai, J.K., Taylor, D.P., 1967. Influence of fungi on host preference host suitability and morphometrics of 5 mycophagous nematodes. Nematologica 13, 529-516.

Ryckeboer, J., Mergaert, J., Coosemans, J;, Deprins, K., Swings, J., 2003. Microbiological aspects of biowaste during composting in a monitored compost bin. J. Appl. Microbiol. 94, 127-137.

Seinhorst, J.W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4, 67-69.

Seinhorst, J.W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178.

Steger, K., Jarvis, A., Smars, S., Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. J. Microbiol. Meth. 55, 371-382.

Steger, K., Sjogren, A.M., Jarvis, A., Jansson, J.K., Sundh, I., 2007. Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste. J. Appl. Microbiol. 103, 487-498.

Termorshuizen, A.J., Moolenaar, S.W., Veeken, A.H.M., Blok ,W.J., 2004. The value of compost. Rev. Environ. Sci. Bio/Technol. 3, 343-347.

Vargas-Garciá, M.C., López, M.J., Suárez-Estrella, F., Moreno, J., 2012. Lignocellulolytic microogranisms from a composting habitat: current and future prospects. In: Pereira,J.C., Bolin, J.L. (Eds.), Composting: processing, materials and approaches. Nova Science Publishers, Inc., New York, pp. 269-294.

Fürst von Lieven, A., Sudhaus, W., 2000. Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. J. Zool. Syst. Evol. Res. 38, 37-63.

Wichuk, K.M., McCartney, D., 2010. Compost stability and maturity evaluation - a literature review. Can. J. Civ. Eng. 37, 1505-1523.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera - an outline for soil ecologists. J. Nematol. 25, 315-331.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fert. Soils 29, 111-129.

Zucconi, F., de Bertoldi, M., 1987. Specifications for solid waste compost. Biocycle 28, 56-61.

5. Survival and colonization of nematodes in a compost process

Modified from:

Steel H, Verdoodt F, Čerevková A, Couvreur M, Fonderie P, Moens T and Bert W. Survival and colonization of nematodes in a compost process. Submitted to Invertebrate Biology.

5.1 ABSTRACT

Nematodes are omnipresent in composts and are active in virtually all stages of the composting process. Major shifts in species composition, life strategies and feeding behavior occur during the composting process. Due to the heat peak, nematodes can be virtually absent, but several taxa appear immediately when the temperatures drop. These comprise both taxa present before the heat peak and new taxa. However, it is completely unknown how nematodes are able to populate the compost. In this paper we aimed to assess the survival and colonization capacity of nematodes in compost. Our results showed that composting processes inaccessible to insects and/or not in contact with soil did not significantly influence the nematode succession during composting. However, for some specific taxa differences between treatments were found (i.e. for *Achrostichus* sp., Neodiplogasteridae sp., *Nygolaimoides* sp. and Rhabditidae sp.1) illustrating the importance of insects for the dispersal of nematodes to compost. Experiments in the lab with the blue bottle fly as a possible carrier demonstrated actual transport of nematodes isolated from compost by the fly (i.e. *Halicephalobus* cfr. *gingivalis*, *Diploscapter coronatus*, *Diplogasteritus* sp., *Acrostichus* sp., *Mesorhabditis* sp.). *Aphelenchoides* sp., *Panagrolaimus* sp. and rhabditid juveniles and dauer stages survived an experimentally induced temperature peak while Tylenchidae did not. In conclusion, our results indicate that the rapidly changing nematode community in a compost process is the result of both differential survival and colonization capacities.

5.2 INTRODUCTION

Nematodes are omnipresent in composts (on average 26 ind./g dry weight compost in Chapter 7) and are active in all stages of the composting process, except during the heat peak where they can be virtually absent (Chapter 2). Major shifts in species composition, life strategies and feeding behavior occur during the composting process. At the beginning of the process (thermophilic phase), immediately after the heat peak, the nematode population is primarily composed of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogasteridae), followed by the bacterial-feeding general opportunists (cp-2) (Cephalobidae) and the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, during the cooling and maturation stages, the bacterial-feeding/predator enrichment opportunistic nematodes (e.g., *Mononchoides composticola*; Chapter 3, part 2) become dominant. Finally, at the most mature stages, the fungal-feeding Anguinidae (mainly *Ditylenchus filimus*), Tylenchidae en

Neotylenchidae are most prominent. This remarkable succession and the distinct advantages of nematodes as bio-indicators (Chapter 1) prompted us to explore the potential of nematodes to assess the maturity and quality of compost (Chapter 2).

However, it is completely unknown how nematodes are able to populate the compost. Although nematodes can be virtually absent during the heat peak, several taxa appear immediately when the temperatures drop. These comprise both taxa present before the heat peak and new taxa. Nematode populations present from the early stages of composting may survive the heat peak as dauer stages or eggs, and/or find refuge in the cooler outer edge of the compost heap. However, other factors must also be important since the nematode community continuously changes during the composting process. New taxa appear after the heat peak, and given that the active mobility of nematodes is in most cases limited to small-scale migrations (Hunt et al. 2001), the question arises as to the origin of these compost colonizers. In general, dispersal by water and wind are thought to be important in generating and maintaining nematode diversity (Hodda et al. 2009). However, many terrestrial nematodes are transported by insects and other mobile invertebrates that share habitat or food resources (Timper and Davies 2004). In this paper we aimed to assess the survival and colonization capacity of nematodes in compost and focused on the following research questions: (1) Does nematode succession differ between a normal, open (control) process and processes that are inaccessible to insects and/or not in contact with soil?, (2) Can vectoring insects effectively be responsible for the transport of nematodes to the composting environment? And (3) are compost nematodes able to survive the temperature increase during the thermophilic phase?

5.3 MATERIALS AND METHODS

5.3.1 Closed vs. non-closed compost processes and its influence on nematode succession

A randomized design with three treatments and three replicates, i.e. nine compost barrels, was set up in a garden in Heusden, Belgium (51° 0'44.5"N; 3° 48' 24.4"E). The barrels had a total volume of 290 l each (height=96 cm, upper diameter=52 cm and lower diameter=80 cm) (Figure 5.1). A first group of three barrels was covered from the soil with plastic canvas, precluding soil organisms from entering the barrels. The second group was covered from soil with plastic canvas and protected with a mosquito net, preventing both soil organisms and insects from reaching the compost. The third group was the control group without any special manipulations affecting insect access and the barrels were placed directly on the soil. These groups will be referred to as

soil cover, net and control treatment, respectively. All barrels were filled with the same amount of feedstock materials on a volume to volume base (v/v): 38 % (v/v) of pure wood chips and 62 % (v/v) of a homogenous mixture. This mixture consisted of 55% fresh grass and 45% mixed wood chips (mainly boxwood and young willow twigs). The compost in the barrels was mixed once, on day 7, during the heat peak. Samples for nematode extraction were taken on the day of the setup (day 0), directly after the heat peak during the thermophilic phase (day 4) and additionally on days 11, 26, 60 and 112. Each sample was a composite sample that consisted of 5 subsamples of 100ml each, taken randomly from different levels in the compost heap. Relevant abiotic variables were measured at every sampling event. For the pH measurements, extractions of 20 g compost in 100 ml distilled water were made, shaken by hand a few times during a 6 hour period, and pH was measured with standard electrodes (Consort P400). Moisture content was determined by weighing 50ml of compost before and after drying for 48h at 104 °C. Total C- and N-content were measured with a Variomax CNS element analyzer, using 1 g of freeze-dried compost (Elementar GmbH, Hanau, Germany), applying the Dumas method (EN[5] 13654-2). Temperature was monitored hourly in the center of the barrels using data-loggers (Testo 175-T2, Ternat, Belgium). To collect insects, traps (pitfalls) were placed in the compost barrel and sticky strips were hung up in and around the barrels. Caught insects were identified up to family level. For an overview of the collected insects see Table 5.S1. These insects were placed on Petri dishes bottom-covered with an agar layer to allow any insect-associated nematodes to colonize the agar (agar medium: 2% bacteriological agar in distilled water (Oxoid, Basinstoke, Uk) and cholesterol (1 µg/ml; Sigma-Aldrich, Belgium), to which *Escherichia coli* OP50 was added as a food source).

[5] EN: European Standard. European Standards are developed by CEN, the European Commission for Standardization. Numbers refer to the specific protocols.

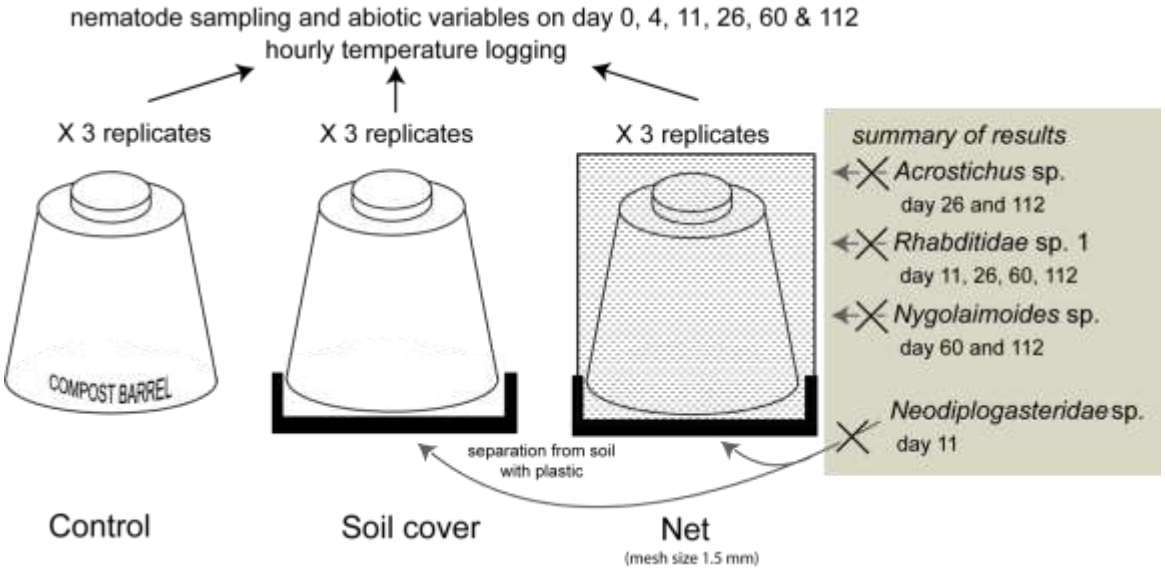


Figure 5.1:
Overview of the experimental setup. Each setup (control, soil cover, net) was replicated 3 times. The 9 barrels were positioned randomly relative to each other and all received the same kinds and amounts of feedstock. In the control group no special manipulations were made to the compost barrel. In the second group (soil cover) the barrels were covered from the soil with plastic canvas, precluding soil organisms from entering the barrel, while in the third group (net) barrels were protected with a mosquito net and covered from soil with plastic canvas, preventing both soil organisms and flying insects from reaching the compost. Compost samples were taken on days 0, 4, 11, 26, 60 and 112 and temperature was measured hourly using data loggers. Nematode species which were absent in the net and/or the soil cover treatments compared to the control are displayed with indication of the days on which they were absent compared to the other treatments.

Nematodes of all 54 400-ml compost samples (3 replicates X 3 treatments X 6 time points) were extracted using the modified Baermann funnel method (tray 49x37.5 cm, basket 38.5x19.5 cm) (Hooper, 1986), counted and 50 individuals (2700 individuals in total) were randomly picked using a stereomicroscope (Leica Mz95). For light microscopy observations the specimens were collected in a very small drop of water in an embryo dish. Formaldehyde (4 % with 1 % glycerol) was heated to 70 °C and an excess (4-5 ml) was quickly added to the specimens to kill and preserve them (Seinhorst 1966). The preserved nematodes were processed to anhydrous glycerol following the glycerol-ethanol method (Seinhorst 1966 as modified by De Grisse 1969) and mounted on mass slides (slide 40x76 mm; cover glass 34x60 mm). Nematodes were identified to genus level. The abundance of each genus or species in each sample was calculated as individuals/gram dry weight compost. Nematode genera were assigned to the 1-5 "coloniser-persister" cp-scale according to their r and K life strategy characteristics (Bongers 1990, 1999), and also classified according to their feeding type (Yeates et al. 1993). These allocations were used to calculate the Maturity Index (MI) (Bongers 1990) and the fungivores/bacterivores ratio (F/F+B ratio) (Yeates et al. 1993). In the F/F+B ratio, only the fungal and bacterial-feeders *sensu*

stricto were included, i.e. bacterial-feeding/predators such as some diplogasterid nematodes were not included. A hierarchical cluster analysis based on Bray-Curtis similarities of square root transformed species abundance data was performed in Primer 6 to assess differences in community composition (Clarke and Warwick 2001). Significant differences in the nematode community between treatments were investigated using Analyses of Similarities (ANOSIM) in Primer 6. Differences in nematode abundances and nematode-based indices (F/F+B and MI) between treatments were tested using non-parametric Kruskal-Wallis and Mann-Whitney U pairwise tests in Statitica 6.0 (Statsoft Inc.) with Bonferroni corrections.

5.3.2 Nematode survival

In a first experiment the temperature tolerance of compost nematodes was tested with monoxenic cultures of nematodes isolated from different composting processes. The used species were: *Diploscapter coronatus*, Rhabditidae sp.1, *Poikilolaimus* sp., *Mesorhabditis* sp. *Acrostichus* sp.1, *Diplogasteritus* sp., *Halicephalobus* cfr. *gingivalis* and *Procephalobus* sp. Three replicates of each culture at or near carrying capacity (with all life cycle stadia present) were placed at 65, 50 and 40 °C for 24h. Afterwards the agar pads of the heat exposed plates were divided in 4 and each piece was transferred to a new plate consisting of solid culture medium (2% bacteriological agar in distilled water; Oxoid, Basinstoke, Uk) with cholesterol (1 µg/ml; Sigma-Aldrich, Belgium), to which *Escherichia coli* OP50 was added as a food source. Nematode recovery (survival and reproduction) was checked once a week for four consecutive weeks.

In the second experiment, 6 subsamples of the samples of day 0 (from the experiment described in 5.2.1) were split in two. One half of the samples was used to extract nematodes using the modified Baermann-funnel method (control) and the other half was first exposed (in an oven) to a temperature peak before nematodes were extracted. This temperature peak imitated the ones that took place in the compost barrels as closely as possible: in the first 12 hours the temperature was raised from 25 to 53° C, then remained constant for 12 hours, and in the following 12 hours was increased to 60 °C, then remaining at that temperature for 24h. Afterwards, during the next three days the temperature was gradually decreased by 10 °C every day. Nematode communities between the control and heat exposed samples were compared based on presence and absence of taxa. However, it should be noted that the used compost samples were stored for over 6 months at 4 °C before the experiment with the induced temperature peak started; of the species present in the original compost material, *Pelodera* sp., Cephalobidae sp. and Neotylenchidae sp. could not be recovered 6 month later at the start of the temperature peak experiment.

5.3.3 Insect phoresy

To further study insect phoresy of compost nematodes, the phoresy of several species by *Calliphora vomitoria* (blue bottle fly) was tested under controlled laboratory conditions. Experiments were carried out in plastic containers, covered with an insect net. They were placed in a closed culture room at a constant temperature (22 °C) and humidity (60%). Each container held 20 adult flies, a donor plate maintaining a monoxenic culture of compost nematodes, and a nematode-free receptor plate. Both donor and receptor plate contained solid culture medium (2% bacteriological agar in distilled water; Oxoid, Basinstoke, Uk) with cholesterol (1 µg/ml; Sigma-Aldrich, Belgium), to which *Escherichia coli* OP50 was added as a food source (Figure 5.2). The densities of nematodes on the donor plates were determined before and after the six-day period. Every 48 hours (T1: 0-48h; T2: 48-96h; T3: 96-144h), receptor plates were checked and replaced by new ones, in order to prevent the reproduction of already transferred nematodes from significantly altering our estimates of the numbers of nematodes transferred by insects. To determine the mean nematode density of the donor plate during the course of the experiment, the average of the pre- and post-experimental density was calculated by quantifying specimens in three defined regions of 0.25 cm^2, distributed evenly on the radius of the circular plate.

This experiment was performed in 2 replicates (R 1 and R 2) with several nematode species isolated from compost. They belonged to three families (Panagrolaimidae: *Halicephalobus* cfr. *gingivalis*; Rhabditidae: *Poikilolaimus* sp., *Diploscapter coronatus*, *Mesorhabditis* sp.; and Diplogasteridae: *Achrostichus* sp., *Diplogasteritus* sp.) and were cultured on solid agar medium. To assess whether flies could have already carried associated nematodes prior to their introduction into our experimental containers, a control treatment was designed without donor plate (control 1). Additionally, a control treatment (control 2) was performed to test for non-fly-dependent transfer of nematodes (e.g. through any circulation of air inside the containers).

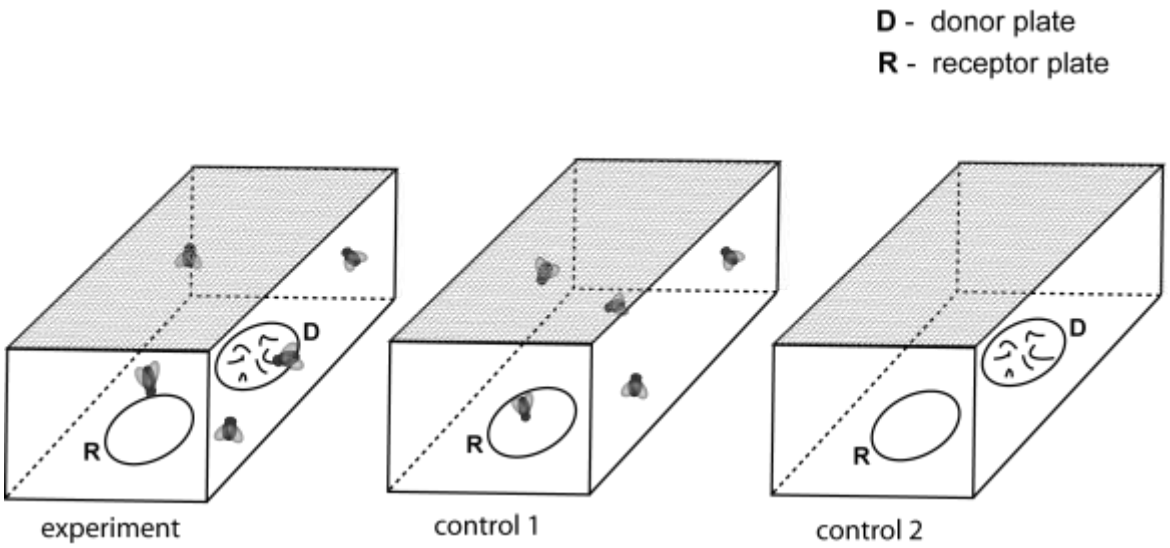


Figure 5.2:
Experimental set-up to test insect phoresy of compost nematodes by *Calliphora vomitoria* (blue bottle fly).

5.4 RESULTS

5.4.1 Closed vs. non-closed compost processes and its influence on nematode succession

Temperature increased quickly after mixing of the feedstock materials in all barrels (Figure 5.3A), exceeding 40 °C within 12 hours and reaching peaks >60 °C within 72 hours. Thereafter, temperature fluctuated probably due to day and night alternation but with an overall gradual decline towards ambient temperatures. The C/N ratio declined from ca. 25 to 15 over 112 days of composting (Figure 5.3B). The pH rose from ca. 5 to 8 during the first 4 days, whereafter it remained fairly constant (Figure 5.3C). Moisture levels remained stable between 20 and 30% (Figure 5.3D).

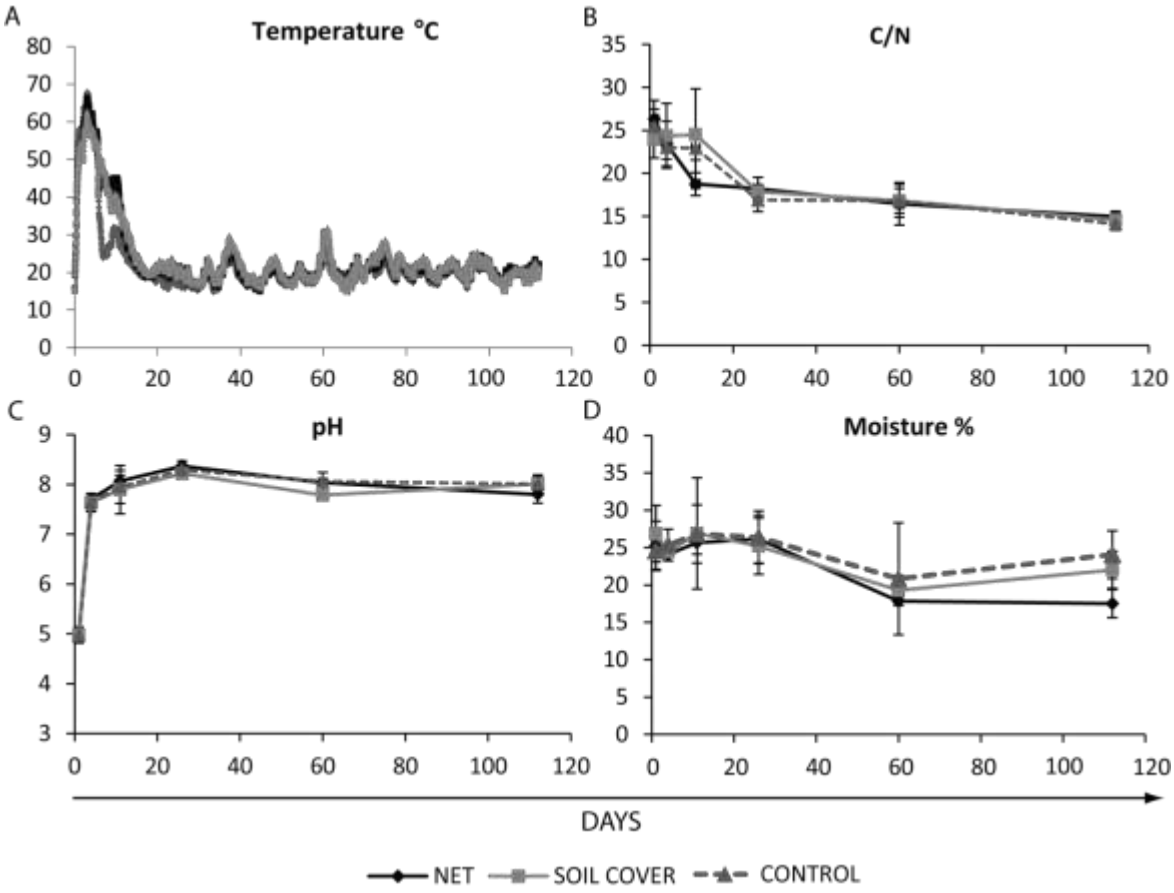


Figure 5.3:
Abiotic variables during the composting process for each of three experimental setups: control, exclusion of soil fauna ('soil cover') and exclusion of both soil fauna and vectoring insects ('net'). (A) Temperature (°C) was measured hourly using data loggers, (B) C/N ratio, (C) pH values, (D) moisture content (%). Data are means of three replicates ± SD.

Irrespective of the experimental setup (control, soil cover or net), very similar patterns in the nematode community were found during the composting process (Figure 5.4 and Table 5.1). For an overview of all nematode taxa found in compost and their feeding type see Table 5.S2. In the feedstock mixture bacterial-feeding and fungal-feeding nematodes were found, making up ca. 80 and 20%, respectively, of the community. During the heat-peak nematodes were absent from all barrels, while on days 11, 26 and 60 there was again a clear dominance of bacterial-feeding nematodes (>80%). On day 112 the proportion of the fungal-feeding nematodes had increased in all setups, though only significantly in the control ($p=0.026$) and soil cover ($p=0.018$) treatment. *Aphelenchoides* sp., *Pelodera* sp., and *Panagrolaimus* sp. were present both before and after the temperature peak, while *Acrostichus* sp.1, Rhabditidae sp.1, *Nygolaimoides* sp. and Neodiplogasteridae sp. were only recorded after the heat peak (Table 5.1). The timing of occurrence and/or presence or absence of the species from the latter group differed between experimental setups (see further). However, overall, differences in nematode community structure were more related to the composting phase than to the treatments including or

excluding nematode migration or transfer. The cluster analysis based on community composition clearly indicated a higher similarity between times of composting than between 'exclusion' treatments (Figure 5.5). Apart from a small cluster (cluster C) which included samples of days 11 and 26 of the control and soil cover treatments, two large clusters could be distinguished: cluster A consisted of all samples from day 0, 11 and 26 and cluster B comprised all samples from day 60 and 112. Furthermore, no significant treatment differences in species composition were found (ANOSIM, $p \geq 0.08$ for all pairwise tests), nor for the nematode abundances or the nematode-based indices (F/F+B and MI) (Figure 5.6).

Nevertheless, the following differences between treatments were observed (Figure 5.1 and Table 5.1). In the control treatment the bacterial-feeding/predators already appeared on day 11, while in the soil cover and net treatment their occurrence was postponed to day 60 and day 112, respectively. Omnivores, i.e. the dorylaimid *Nygolaimoides* sp., were found on days 60 and 112 in control and on day 112 in the soil cover treatment but were absent from the net treatment. Rhabditidae sp.1 was found from day 11-112 in control and soil cover treatments but never in net treatments. Neodiplogastridae sp. was present exclusively on day 11 in the control treatment and *Acrostichus* sp.1 occurred from day 26-112 in the control and soil cover treatment but only on day 66 in the net treatment.

Only one nematode taxon could be retrieved from the Petri dishes with insects collected using insect-traps in the compost bins. The genus *Acrostichus* was isolated from insects (3) from the Sciaridae family (Order Diptera). However, this species was clearly different from the *Acrostichus* population in the compost.

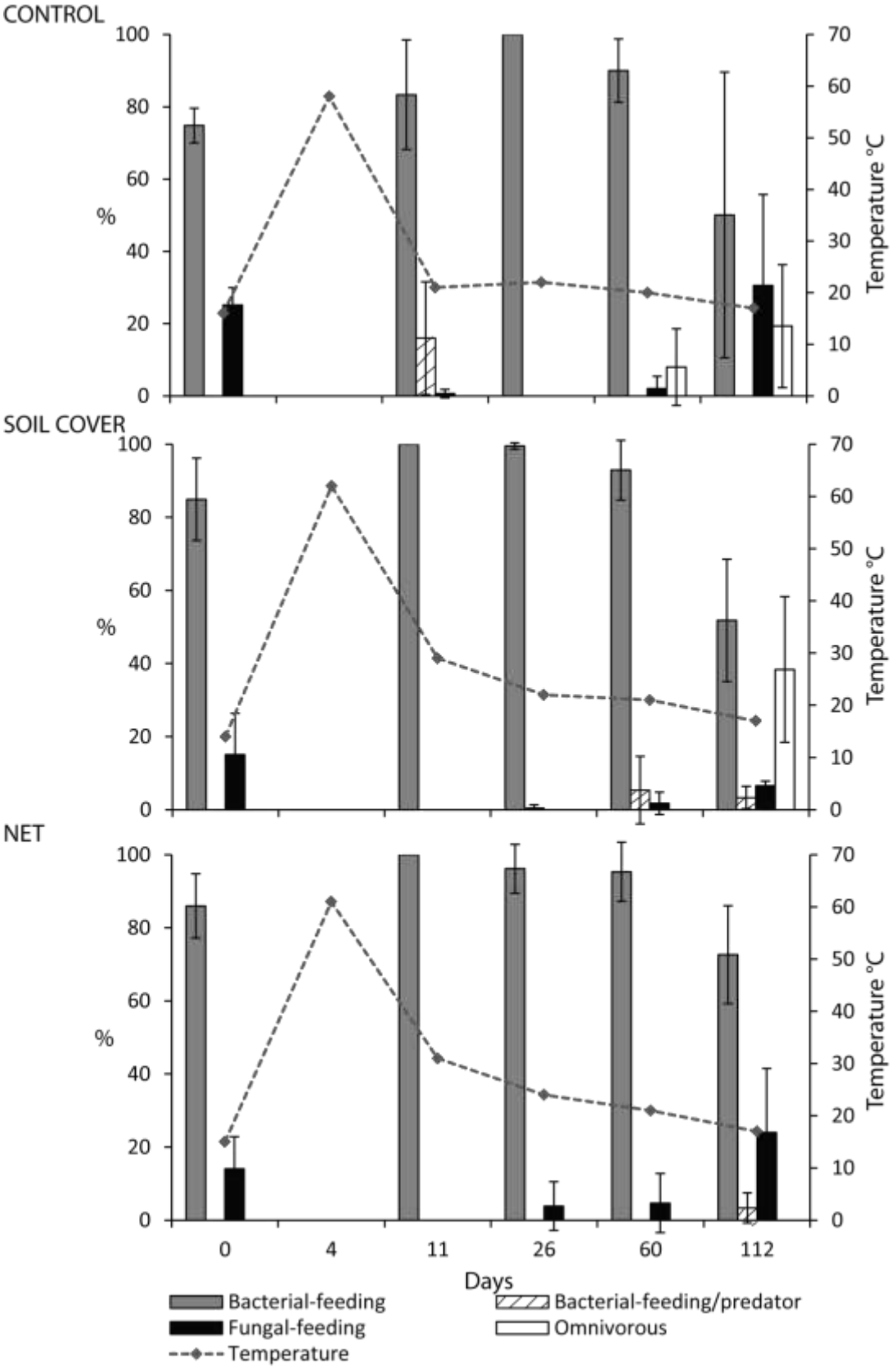


Figure 5.4:

The relative contributions of each nematode feeding type (bacterial-feeding, fungal-feeding, bacterial-feeding/predator and omnivores) at every sampling moment. Data are means of three replicates ± SD. Mean temperature (°C) at the time of sampling is indicated on the second Y-axis as a line.

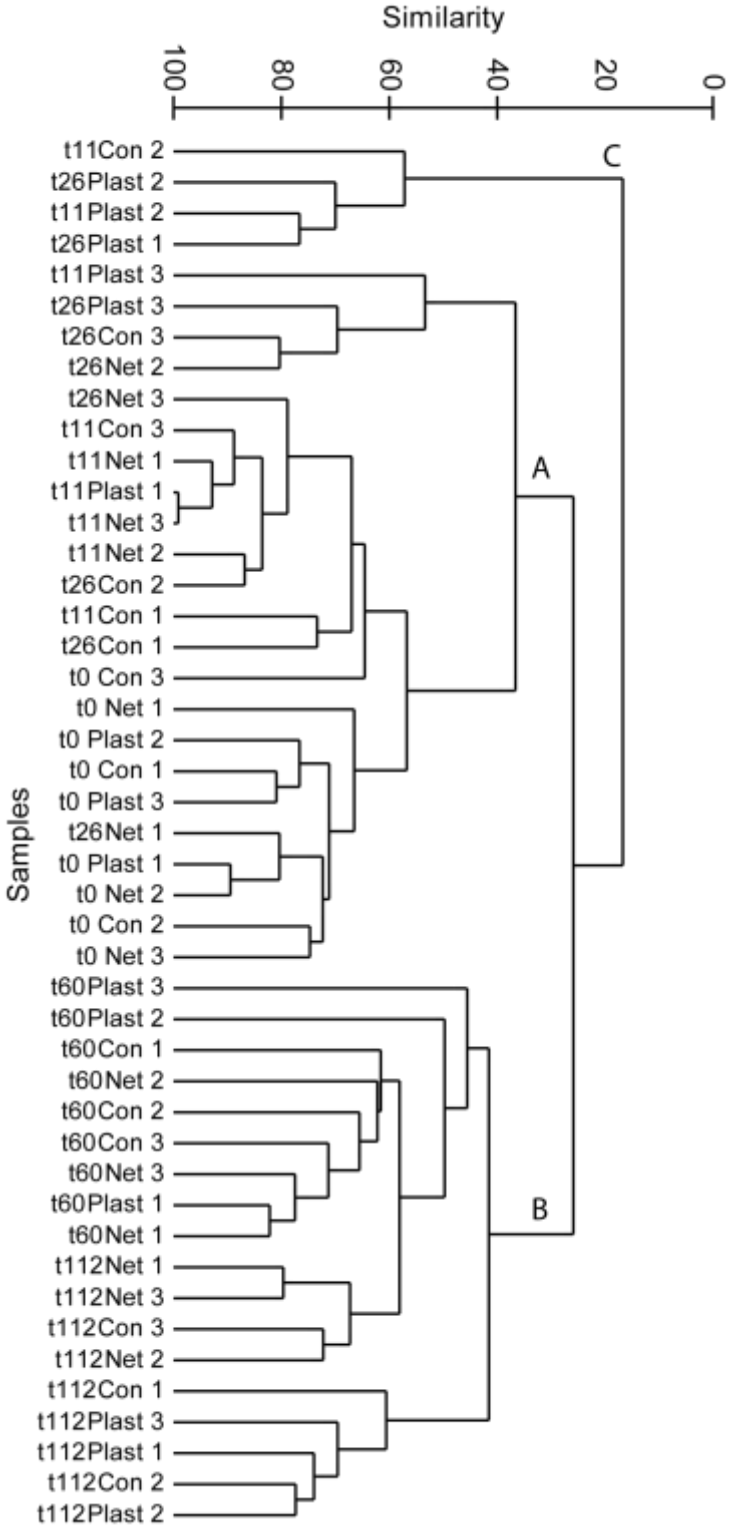


Figure 5.5:

Hierarchical clustering of all nematode samples of all treatments, using group-average linking of Bray-Curtis similarities calculated on square-root transformed abundance data. The B cluster consists of samples of day 60 and 112 while the A and C cluster consist of earlier samples of days 0-26 (except for day 4 when nematodes were absent). In the labels of the samples "Con" refers to samples from the control group where no special manipulations were made to the compost barrel, "Plast" refers to samples from the soil cover treatment where the barrels were covered from the soil with plastic canvas, while "Net" refers to the net treatment samples where barrels were protected with a mosquito net and covered from soil with plastic canvas.

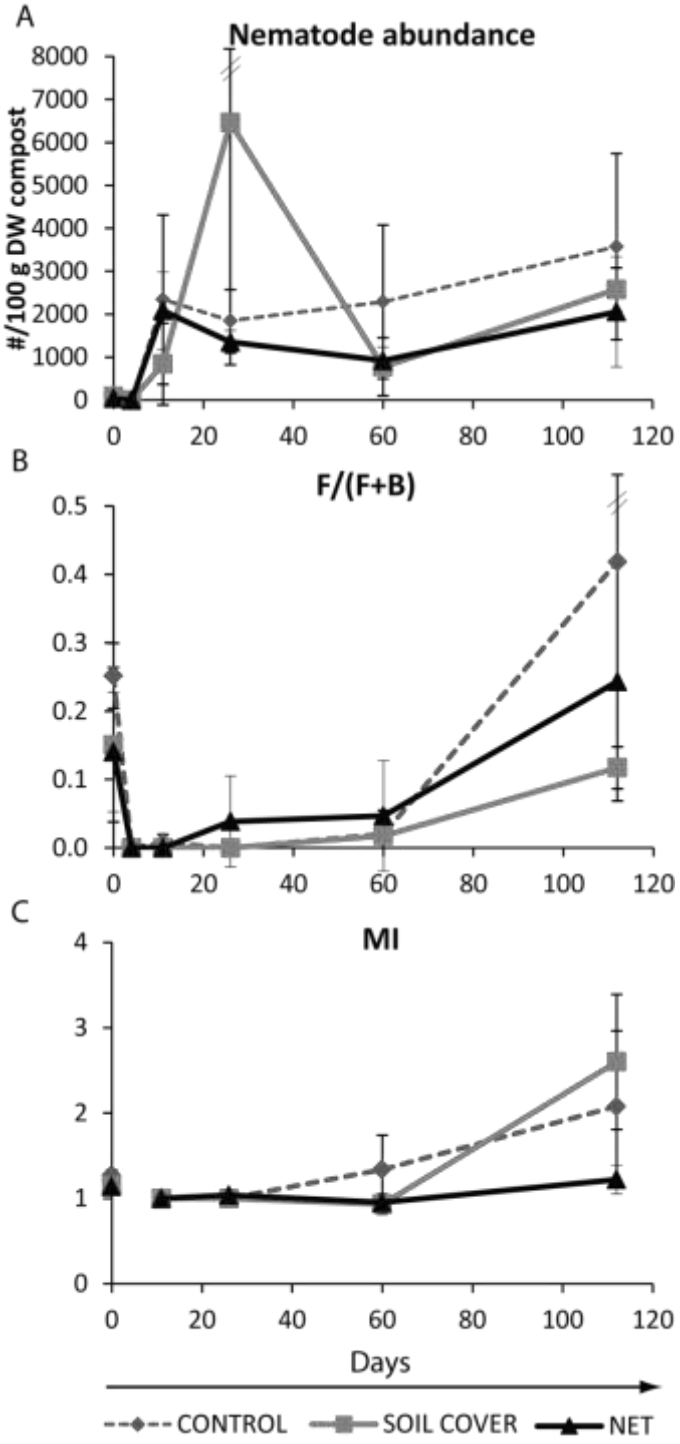


Figure 5.6:
Overview per treatment (control, soil cover and net) of (A) total nematode abundance (#/100 g DW compost) during composting and the nematode based indices: (B) F/F+B ratio based on the fungal- and bacterial-feeding (*s.s.*) densities, (C) Maturity Index (MI) based on the allocation of nematode taxa to cp-groups,. In (C) MI could not be calculated for day 4 because of the absence of nematodes at this time. Values are means ± SD based on three replicates.

Table 5.1:
Overview of the mean species abundance during the different composting phases, i.e. in the feedstock mixture (day 0), during cooling (day 11 + 26) and during maturation (day 60 +1 1). During the heat peak (day 4) no nematodes were found and therefore this phase is not included in the table. Data are means per composting phase ± SD.

	Start (Day 0)			Cooling (Day 11 + 26)			Maturation (Day 60 + 112)		
	control	soil cover	net	control	soil cover	net	control	soil cover	net
Acrostichus sp.	0 ± 0	0 ± 0	0 ± 0	43 ± 53	160 ± 392	0 ± 0	160 ± 263	70 ± 96	25 ± 37
Aphelenchoides sp.	8 ± 5	6 ± 5	5 ± 4	13 ± 32	0 ± 0	20 ± 50	391 ± 446	75 ± 84	169 ± 214
Bunonema sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 24	11 ± 19
Cephalobidae sp.	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Diplogasteridae juv.	0 ± 0	0 ± 0	0 ± 0	71 ± 173	1699 ± 3469	127 ± 311	0 ± 0	5 ± 12	38 ± 48
Diploscapter sp.	0 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1321 ± 1540	391 ± 453	747 ± 890
Ektaphelenchoides sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	59 ± 144	37 ± 66
Halicephalobus sp.	0 ± 1	0 ± 1	0 ± 0	0 ± 0	132 ± 234	10 ± 23	155 ± 179	181 ± 210	177 ± 98
Mononchoides sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	62 ± 74	52 ± 113
Neodiplogastridae sp.	0 ± 0	0 ± 0	0 ± 0	91 ± 130	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Neotylenchidae sp.	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 24	0 ± 0
Nygolaimoides sp.	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	380 ± 438	513 ± 691	0 ± 0
Panagrolaimus sp.	4 ± 4	24 ± 23	15 ± 14	13 ± 32	2 ± 5	61 ± 115	0 ± 0	0 ± 0	0 ± 0
Pelodera sp.	8 ± 6	21 ± 32	10 ± 6	952 ± 992	342 ± 553	411 ± 260	66 ± 103	49 ± 94	12 ± 28
Plectidae juv.	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Prismatolaimus sp.	0 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Rhabditidae	9 ± 3	35 ± 37	7 ± 3	881 ± 555	231 ± 524	1090 ± 703	380 ± 323	73 ± 92	220 ± 137
Rhabditidae sp. 1	0 ± 0	0 ± 0	0 ± 0	17 ± 41	1084 ± 2206	0 ± 0	78 ± 121	169 ± 183	0 ± 0
Tylenchidae sp.	0 ± 0	0 ± 1	0 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 24	0 ± 0

5.4.2 Nematode survival

None of the tested species recovered from a 24 h temperature increase to 50 or 65 °C within 4 weeks (experiment 1). After the temperature increase to 40 °C only *Halicephalobus* cfr. *gingivalis* and *Procephalobus* sp. could be retrieved. However, when compost samples of day 0, comprising *Panagrolaimus* sp., *Aphelenchoides* sp., Tylenchidae sp. and Rhabditidae sp. (juveniles and dauer stages), were exposed to an experimental temperature peak (up to 60 °C), *Panagrolaimus* sp., *Aphelenchoides* sp. and Rhabditidae sp.1 were able to survive in respectively all, 5 and 5 out of 6 replicates. Hence, only Tylenchidae sp. did not survive the heating up to 60° of compost samples.

5.4.3 Insect phoresy

After 48 hours, transfer of nematodes from donor to receptor plate (Figure 5.2) was observed for *Halicephalobus* cfr. *gingivalis*, *Diploscapter coronatus*, *Poikilolaimus* sp. (only second replicate), *Diplogasteritus* sp. and *Acrostichus* sp. Phoresy of *Mesorhabditis* sp. was first observed after 72 hours. *Poikilolaimus* sp. were never observed on the receptor plate in the first replicate, nor were any nematodes in both control experiments. For each species, the potential of phoresy was scored, based on the total number of transferred nematodes (T1 + T2 + T3), relative to the mean density of that species on the donor plate (Table 5.2). The successful

transportations of *Halicephalobus* cfr. *gingivalis* (in both replicates) and of *Mesorhabditis* sp. and *Poikilolaimus* sp. (in the second replicate) by the flies exceeded that of the other species by a factor 10 (except for *Diploscapter* sp. and *Diplogasteritus* sp. in the first replicate). Our observations indicated transfer by the flies of both adults and juveniles, except for *Diploscapter coronatus* and *Acrostichus* sp. where only juveniles were transported.

Table 5.2:
Table representing the potential of phoresy for tested compost nematodes by *Calliphora vomitoria* (blue bottle fly) for the first replicate (R 1) and the second replicate (R 2). Transport by the flies was observed with the highest success rate for *Halicephalobus* cfr. *gingivalis*, *Poikilolaimus* sp. and *Mesorhabditis* sp. (in respectively both, R 2 and R 2) and also for *Mesorhabditis* sp., *Diploscapter coronatus*, *Diplogasteritus* sp. and *Acrostichus* sp. but never for *Poikilolaimus* sp. in the first replicate. In the two control treatments no nematodes were found on the receptor plates.

		R 1	R 2
Controls	Control 1	-	-
	Control 2	-	-
Panagrolaimidae	*Halicephalobus* cfr. *gingivalis*	++	++
Rhabditidae	*Mesorhabditis* sp.	+	++
	Diploscapter coronatus	+	+
	Poikilolaimus sp.	-	++
Diplogasteridae	*Diplogasteritus* sp.	+	+
	Acrostichus sp.	+	+

++ ≥ 1% transferred

+ ≤ 0.5% transferred

- no transfer

5.5 DISCUSSION

The treatments with different possibilities for compost colonization had a limited effect on the overall nematode succession patterns. For some specific taxa, however, the soil cover and/or net treatment produced an effect, sometimes as a delayed colonization, i.e. taxa arrived later than in the control treatment. Some caution is due when interpreting these relatively minor differences between treatments. For instance, we noted very slight damages to the insect nets near the end of the experiment, so we cannot completely exclude that some small Diptera could have been able to enter the net treatment in the later stages of the experiment. Also, some insect eggs or larvae could have been present in the feedstock material at T_0 and could have potentially contained and later released nematodes.

Inspite of these small treatment differences, the overall nematode succession in all 9 barrels was remarkably comparable with that of previous studies focusing on considerably larger-scale outdoor composting processes with greatly different feedstock materials (Chapter 2, 4 and 6). However, in these former studies, the bacterial-feeding/predators were more prominently present in the early cooling phase, which was less pronounced in the control of the present experiment and delayed in the soil cover and net treatment (Figure 5.4). The recurrence of the nematode succession is a strong indication that the described nematode patterns are universal for compost, and are relatively independent of the environmental conditions of the composting process, since covering or closing from insects or soil has very little effect. The scale of composting, which was more than 10-fold smaller in the barrels compared to the large-scale processes of the previous studies, also did not really affect this typical nematode succession. In addition, feedstock composition did not show a pronounced effect on the nematode community in mature compost (Chapter 7), and the nematode succession was not influenced by the season of composting since processes were run from spring to summer in this study compared to in autumn-winter in chapter 2 and 4.

Nevertheless, in spite of the generally very similar nematode communities between treatments, differences were observed. These include complete absence of *Nygolaimoides* sp. and Rhabditidae sp.1 , and, albeit only temporarily, of *Acrostichus* sp. (i.e. present in control and soil cover treatment for day 26-112 but only in net on day 66) in the net treatment, and of Neodiplogasteridae sp. in the soil cover and net treatment. The dorylaim *Nygolaimoides* sp. was found in consecutive samplings (i.e. day 60 and 112) in the control setup and in the three replicates of only the last time point (i.e. day 112) in the soil cover setup. In previous composting processes (Chapter 2, 4 and 6) dorylaims were only sporadically found. They are typical K-strategists and tend to be very sensitive to different types of disturbances (Bongers 1999). Here,

in the dynamic composting environment, they made up an important part of the community, between 4-32 and 24-61% respectively, during the second half of our experiment. *Nygolaimoides* sp. thus survived and reproduced in the compost environment, and their absence from the net treatment indicates that they are transported by (flying) arthropods. To the best of our knowledge, the only previous report of a dorylaim with a phoretic relationship with an arthropod vector is that of *Nygolaimoides borborphilus* transported by dung beetles (Sachs 1950) and Diptera (Chizhov 1996). Although dorylaims are highly abundant and diverse and have been suggested to play an important role in soil food webs (Peña-Santiago 2006; Khan and Kim 2007), their ecology has been far less studied compared to e.g. rhabditids, partly as a result of the difficulty at maintaining or culturing them under lab conditions. The absence of Neodiplogasteridae in both the net and the soil cover treatment indicates that also soil dwelling animals (e.g. a non-flying beetle) could be involved in nematode phoresy. For the diplogasterid *Acrostichus* sp., another taxon of which the colonization was temporarily retarded by the net treatment, we have demonstrated actual transport by an insect; albeit in this case a flying one. The experiments with *Calliphora vomitoria* confirmed that 5 out of 6 tested compost nematodes (i.e. *Halicephalobus* cfr *gingivalis, Mesorhabditis* sp., *Diploscapter coronatus*, *Diplogasteritus* sp., *Acrostichus* sp.) were effectively able to reach a new habitat (i.e. culture plate) by hitching a ride on flies, with *Halicephalobus* cfr. *gingivalis* being the most efficient hiker. However, since colonization differences between our treatments could not be observed for *Halicephalobus* cfr. *gingivalis*, other dispersal mechanisms could also play a role. Our results are a confirmation that insect phoresy is widespread among opportunistic colonizers (Timper and Davies 2004; Bongers 1999) like diplogasterids (e.g. *Diplogaster corprophila* (Kiontke 1996), *Acrostichus rhynchophori* (Kanzaki et al. 2009b)), panagrolaimids (e.g. *Panagrellus leperisini* and *P. ludwigi* Massey 1974 in Stock and Nadler 2006) and rhabditids (e.g. several *Caenorhabditis* species (Sudhaus and Kiontke 1996), *Rhabditis stammeri* (Richter 1993) and *Poikilolaimus floridensis* (Kanzaki et al. 2009)). It is especially these groups that are prominently present during the composting process. Current study on a compost process demonstrated, based on both in vivo and in vitro experiments, that colonization by a relatively diverse nematode community is only a matter of weeks. Our experimental evidence thus indicates the potential of soil dwelling organisms such as nematodes to disperse over a relatively long distance in a very short time. This strongly endorses the ubiquity hypothesis for microscopic organisms, i.e. ‘everything is everywhere, but the environment selects’ (Fenchel and Finley 2004). This explains for instance the widespread distribution of some of the tested nematodes such as *Diploscapter coronatus*. This is also in agreement with Wardle et al. (2003) who stated, in the framework of island ecology, that the

physical distance of suitable habitats to sources of colonizing organisms is not an important factor for the structure of decomposer communities. In agreement with Kruitbos et al. (2009) our results demonstrated that nictation (i.e. waving behavior of nematodes) is not a prerequisite for phoresis, nematodes such as *Halicephalobus* cfr. *gingivalis* could be transported by the fly but did not nictate. However, in many cases the phoretic relation is much more complex as transport can be internal and external, passive or active, and very often associations between nematodes and insects are highly species-specific (e.g. Krishnan et al. 2010).Since phoretic relations between nematodes and insects are common in compost, and as it is considered an important evolutionary step towards parasitism (Dillman et al. 2012), compost might be the perfect habitat to find an undiscovered diversity of insect associated nematodes. Thus, as previously suggested for micro-organisms (Hoitink and Boehm 1999), compost might be used as an organic carrier for antagonists such as nematodes with bio-control properties.

Next to colonization of the compost by insects we have demonstrated that survival of nematodes during compost heat peaks is possible, even for temperature peaks up to 60°C. This is much higher than expected based solely on temperature limits of nematodes that are based on simple lab experiments. Since a lot of juveniles could be recovered directly after the induced heat peak, these nematodes may survive as dauer stages or eggs. The specific structure of compost material, including the presence of micro-niches, each with slightly different conditions, might provide a more protected environment compared to agar cultures, e.g., in maintaining adequate moisture levels at high temperatures. The precise mechanism responsible for nematode survival in compost, however, remains to be unraveled. For instance, controlling the moisture level in agar plates could indicate the importance of moisture in survival of compost nematodes. Both our field and lab experiment indicate that both *Aphelenchoides* sp. and *Panagrolaimus* sp. are able to survive higher temperatures in a compost environment, as they were present both before and immediately after the (induced) heat peak.

In conclusion, our results indicate that the rapidly changing nematode community in a compost process is the result of both differential survival and colonization capacities. For several taxa we could readily explain their presence in compost by evidence of survival of the heat peak (i.e. *Aphelenchoides* sp., *Panagrolaimus* sp. and Rhabditidae in general) or for colonization using insects as carriers (i.e. *Acrostichus* sp., Neodiplogasteridae sp., *Nygolaimoides* sp., Rhabditidae sp1.). Although no proof was found in the experiment with the compost barrels, the experiments with the blue bottle fly as possible carrier indicated that insects may also play an important role in the dispersal of *Diploscapter coronatus* and *Halicephalobus* cfr. *ginigvalis*

whether it is to compost or to other habitats. However, the colonization of a compost process by several other taxa (Table 5.3) remains to be investigated.

Table 5.3:

Schematic overview of the nematode species during the composting process in the three treatments (C= control, SC= soil cover, N= net), with indication of experimental evidence for survival of the heat peak or colonization of the compost after the heat peak.

	Start Day 0			Cooling Day 11 + 26			Maturation Day 60 + 112			Evidence for survival	Evidence for colonization
	C	SC	N	C	SC	N	C	SC	N		
Acrostichus sp.											X
Aphelenchoides sp.										X	
Bunonema sp.											
Cephalobidae sp.											
Diplogasteridae juv.											
Diploscapter sp.											X*
Ektaphelenchoides sp.											
Halicephalobus sp.											X*
Mononchoides sp.											
Neodiplogasteridae sp.											X
Neotylenchidae sp.											
Nygolaimoides sp.											X
Panagrolaimus sp.										X	
Pelodera sp.											
Plectidae juv.											
Prismatolaimus sp.											
Rhabditidae										X	
Rhabditidae sp. 1											X
Tylenchidae sp.											

* only evidence for possible transport by the blue bottle fly but not from the experiment with the different treatments in the barrels

only in feedstock
both before and after heat peak
only after heat peak

5.6 SUPPLEMENTARY MATERIAL

Table 5.S1

Overview of the insect families that frequent the compost barrels during the course of the experiment. A distinction was made between insects caught with pitfalls and insects collected with sticky strips.

	pitfall	sticky strip
Acari	X	
Anisopodidae	X	
Anthicidae	X	
Calliphora vicina		X
Chironomidae	X	
Chrysomelidae	X	
Collembola	X	
Coccinellidae		X
Cynipidae	X	
Dolichopodidae		X
Drosophila sp.		X
Dytiscidae	X	X
Formicidae	X	
Hybotidae	X	
Ichneumonidae	X	X
Monotoma picipes	X	
Muscidae		X
Phoridae	X	
Psilidae	X	
Sarcophagidae		X
Scatopsidae	X	
Sciaridae	X	X
Sphaeroceridae	X	
Sphecidae		X
Staphylinidae	X	X
Syrphidae		X
Tenthredinidae		X
Coleoptera larvae	X	
Muscidae larvae	X	
Diptera larvae	X	
Stratiomyidae larvae	X	

Table 5.S2

Overview of the nematode taxa found in compost and their feeding type mainly based on Yeates et al. (1993)

TAXA	Feeding type
***Achrostichus* sp.**	3
***Aphelenchoides* sp.**	2
***Bunonema* sp.**	3
***Butlerius* sp.**	3-5a
***Cephaloboides* sp.**	3
***Cephalobus* sp.**	3
***Choriorhabditis* sp.**	3
***Crusthorhabditis* sp.**	3
***Cruznema* sp.**	3
***Diplogaster* sp.**	3-5a
***Diplogasteritus* sp.**	3
***Diplogasteroides* sp.**	3
Diploscapter coronatus	3
Ditylenchus filimus	2
***Ektaphelenchoides* sp.**	/
***Eucephalobus* sp.**	3
***Fictor* sp.**	3-5a
***Filenchus* sp.**	2
Halicephalobus* cfr. *gingivalis	3
***Mesorhabditis* sp.**	3
Mononchoides composticola	3-5a
Neotylenchidae unidentified sp.	2
***Nygolaimoides* sp.**	8
***Panagrellus* sp.**	3
***Panagrolaimus labiatus* sp.**	3
***Parasitorhabditis* sp.**	3
Pelodera cylindrica	3
Pelodera teres	3
***Poikilolaimus* sp.**	3
***Procephalobus* sp.**	3
***Protorhabditis* sp.**	3
Rhabditella axei	3
Rhabditophanes cobbi	3
***Seinura* sp.**	5
***Teratorhabditis* sp.**	3

5.7 REFERENCES

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technol. 74, 35-47.

Bailey, K.L., Lazarovits, G., 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil Till. Res. 72, 169-180.

Baldwin, K.R., Greenfield, J.K. Composting on organic farms. Publication no. AG-659W-01. 2006. North Carolina Cooperative Extension Service, NC State Univeristy. Organic production.

Bongers, T., 1990. The Maturity Index - an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Bongers, T., 1999. The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. Plant Soil 212, 13-22.

Butler, T.A., Sikora, L.J., Steinhilber, P.M., Douglass, L.W., 2001. Compost age and sample storage effects on maturity indicators of biosolids compost. J. Environ. Qual. 30, 2141-2148.

Chizhov, V.N., 1996. Phoresia of *Nygolaimoides borborophilis* (Nematoda/ Dorylaimida) by scatobiotic dipterans. Russ. J. Nematol. 4, 185-186.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth.

Cogger, C.G., 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Sci. Util. 13, 243-251.

De Grisse, A.T., 1969. Redescription ou modifications de quelques technicques utilisées dans l' étude des nématodes phytoparasitaires. Meded. Rijsfak. Landbouwwet Gent, 34, 351-369.

Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. PLoS Pathog. 8, 1-4.

Fenchel, T., Finlay, B.J., 2004. The ubiquity of small species: patterns of local and global diversity. Bio. Science. 54: 777–784.

Gamliel, A., Austerweil, M., Kritzman, G., 2000. Non-chemical approach to soilborne pest management - organic amendments. Crop Prot. 19, 847-853.

Hodda, M., Peters, L., Traunspurger, W., 2009. Nematode diversity in terrestrial, freshwater aquatic and marine systems. In: Wilson, M.J., Kakouli-Duarte, T. (Eds.), Nematodes as Environmental Indicators. CABI, Cambridge, pp. 45-93.

Hooper, D.J., 1986. Extraction of free-living stages from soil. In: Southey, J.F. (Ed.), Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 5-30.

Hunt, H.W., Wall, D.H., Decrappeo, N.M., Brenner, J.S. 2001. A model for nematode locomotion in soil. Nematology 3, 705-716.

Ingham, E.R., Slaughter, M.D. , 2004. The soil foodweb- soil and compost as living ecosystems. I International Conference Soil and Compost Eco-Biology, León, Spain.

Kanzaki, N., Giblin-Davis, R.M., Scheffrahn, R.H., Center, B.J., 2009a. *Poikilolaimus floridensis* n. sp. (Rhabditida: Rhabditidae) associated with termites (Kalotermitidae). Nematology 11, 203-216.

Kanzaki, N., Giblin-Davis, R.M., Zeng, Y., Ye, W., Center, B.J., 2009b. *Acrostichus rhynchophori* n. sp. (Rhabditida: Diplogastridae): a phoretic associate of *Rhynchophorus cruentatus* Fabricius and *R. palmarum* L. (Coleoptera: Curculionidae) in the Americas. Nematology 11, 669-688.

Kiontke, K., 1996. The phoretic association of *Diplogaster coprophila* Sudhaus & Rehfeld, 1990 (Diplogastridae) from cow dung with its carriers, in particular flies of the family Sepsidae. Nematologica 42, 354-366.

Krishnan, A., Muralidharan, S., Sharma, L., Borges, R.M., 2010. A hitchhiker's guide to a crowded syconium: how do fig nematodes find the right ride? Funct. Ecol. 24, 741-749.

Kruitbos, L.M., Heritage, S., Wilson, M.J., 2009. Phoretic dispersal of entomophatogenic nematodes by *Hylobius abietis*. Nematology 11, 419-427.

Kuo, S., Ortiz-Escobar, M.E., Hue, N.V., Hummel, R.L., 2004. Composting and compost utilization for agronomic and container crops. Recent Res. Devel. Environ. Biol. 1, 451-513.

Kutzner, H.J., 2000. Microbiology of composting. In: Rehm, H.J., Reed, G. (Eds.), Biotechnology. Wiley-VCH, pp. 35-100.

Peña-Santiago, R., 2006. Dorylaimida part I: Superfamilies Belondiridae, Nygolaimoidea and Tylencholaimoidea. In: Eyualem-Abebe, Andrássy, I., Traunspurger, W. (Eds.), Freshwater nematodes: ecology and taxonomy. CABI, Oxfordshire, UK, pp. 326-391.

Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, S., Bar, A., Orion, D., 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Prot. 21, 583-587.

Richter, S., 1993. Phoretic association between the dauerjuveniles of *Rhabditis stammeri* (Rhabditidae) and life history stages of the burying beetle *Nicrophorus vespilloides* (Coleoptera: Silphidae). Nematologica 39, 346-355.

Sachs, H.G., 1950. Die nematodenfauna der rinderexkremente. Zool. Jahrb. (Syst) 78, 323-366.

Seinhorst, J.W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178.

Stock, S.P., Nadler, S.A., 2006. Morphological an molecular characterization of *Panagrellus* spp. (Cephalobina: Panagrolaimidae): taxonomic status and phylogenetic relationships. Nematology 8, 921-938.

Sudhaus, W., Kiontke, K., 1996. Phylogeny of Rhabditis subgenus *Caenorhabditis* (Rhabditidae, Nematoda). J. Zool. Syst. Evol. Res. 34, 217-233.

Timper, P., Davies, K.G., 2004. Biotic interactions. In: Gaugler, R., Bilgrami, A.L. (Eds.), Nematode Behaviour. CAB International, Wallingford, UK, pp. 277-307.

Vallad, G.E., Cooperband, L., Goodman, R.M., 2003. Plant foliar disease suppression mediated by composted forms of paper mill residuals exhibits molecular features of induced resistance. Physiol. Mol. Plant P. 63, 65-77.

Wardle, D.A., Yeates, G.W., Barker, G.M., Bellingham, P.J., Bonner, K.I., Williamson, W.M., 2003. Island biology and ecosystem functioning and epiphytic soil communities. Science 301, 1717-1720.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera - An outline for soil ecologists. J. Nematol. 25, 315-331.

Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biol. Fert. Soils 37, 199-210.

Zucconi, F., de Bertoldi, M., 1987. Specifications for solid waste compost. Biocycle 28, 56-61.

6. Factors influencing the nematode community during composting: comparison between experimental farm compost and industrial green waste compost

Publication in preparation.

6.1 ABSTRACT

According to pilot studies, shifts in the nematode species composition, life strategies and feeding behavior occur during the composting process. Although these shifts appear to be fairly consistent and therefore most promising as a potential tool to assess compost maturity, they are hitherto only based on a limited number of mainly non-replicated observations. In this study we tested if the nematode community succession patterns are recurrent for similar but independent processes and assess the relation between the changes in the nematode community and potential important variables (i.e. temperature, duration of composting and the microbial community). Therefore the nematode and microbial community of three simultaneously running controlled farm composting processes were analyzed through time and compared with a single industrial green waste composting process. Persistent high temperatures in this standard industrial process resulted in very low nematode abundances and aberrant nematode succession patterns, including the virtual absence of a fungal-feeding population. The general patterns of the controlled and well balanced farm compost were largely similar to the previous studies, i.e. during and directly after the heat peaks bacterial-feeding enrichment opportunists are most important. Subsequently, the bacterial-feeding/predator community dominated and during maturation the fungal-feeding nematodes became more important. However, the timing of the nematode succession in the farm compost processes was different compared to previous studies, with a greater fluctuation of fungivorous/bacterivorous indices. This is likely due to the fact that temperature in these processes did not show a simple decrease with time as in the other studies. This, however, allowed us to better distinguish between the effect of time and temperature in a generalized linear mixed model. The nematode abundances were significantly related with temperature; the amount of fungal-feeding nematodes was significantly related with both duration of composting and temperature, and the F/F+B ratio was only significantly related with duration of composting. Addition of PLFA based data (*i.e.* fungal PLFA or PLFA based F/B ratio) as a variable in the appropriate models did not significantly add to the explanation of the observed data. As shown, the nematode succession in monitored compost processes follows a repeatable and predictable pattern and especially the nematode based F/F+B holds promise as a reliable indicator of compost maturity since it was not significantly affected by temperature fluctuations but mainly related with duration of composting.

6.2 INTRODUCTION

Composting is an aerobic, heat producing and controlled process in which microorganisms convert a mixed organic substrate into carbon dioxide (CO_2), water, inorganic nutrients and stabilized organic matter. Compost can be used as a fertilizer (Cogger 2005; Zvomuya et al. 2008) and is also known as a suppressive agent against plant diseases (e.g. Akthar and Malik 2000; Kuo et al. 2004). The taxa that are most important to the composting process are bacteria, algae, fungi, isopoda, acari, nematoda and protozoa (Cooperband 2000; Young et al. 2005). This wide spectrum of organisms forms a complex and rapidly changing community. Of all these taxa only nematodes (Chapter 2 and 4) and bacteria (Ryckeboer et al. 2003; Chapter 4) are ubiquitous in all stages of the compost process, making them the most important groups to analyze during the composting process. Furthermore the nematode community is considered to feed mainly on the microbial community and shows several advantages over the latter rendering nematodes more suitable bio-indicators of ecosystem processes and quality (Neher 2001). For example nematodes are more integrated in the food web by being one or two steps higher and their response to changes are more significant on a stable temporal scale because of their longer generation time.

According to pilot studies (Chapter 2 and 4) a shift in nematode species composition, life strategies and feeding behavior occurs during the composting process. At the beginning of the process, during the thermophilic phase, the nematode population is primarily built by bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogasteridae), followed by the bacterial-feeding general opportunists (cp-2) (Cephalobidae) and the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, via a community with increasing numbers and dominance of bacterial-feeding/predators (Neodiplogasteridae mainly *Mononchoides composticola*) in the cooling phase, finally the fungal-feeding general opportunists (Anguinidae and Aphelenchoididae) become more important during the maturation phase. And this increasing proportion of fungal-feeding nematodes during the compost process was proposed as a potential indicator of compost maturity[6] (see Chapter 2 and 4). Compared to nematode community, the microbial community, as revealed by phospholipid fatty acids (PLFA), showed an earlier shift towards higher proportions of fungi during both cooling and maturation phases. The delay of fungal-feeding nematodes with respect to the microbial community was mainly ascribed to the dominance of the bacterial-feeding/predators during cooling and to the relatively long generation time of most fungal-feeding nematodes.

[6] For more information on and definition of compost maturity see 1.3.

Although the nematode community shifts appear to be fairly consistent and most promising as a potential tool to assess compost maturity, these patterns are hitherto only based on a limited number of observations (single case non-replicated processes) and based on the same method of composting (i.e. on farm Controlled Microbial Composting (CMC), referred to as farm compost). More or less similar community patterns as in the large scale farm composting systems were observed in short term, small scale processes in compost barrels (Chapter 5). Furthermore, although it is suggested that these remarkable nematode composition shifts are related to processing time, compost temperature and/or microbial community (Chapter 2 and 4), a replicate design is warranted to have a better insight in the underlying factors that might be related with the changing nematode community in compost.

In this study, the nematode and microbial community of three simultaneously running controlled farm compost processes, with different proportions of feedstock materials, were analyzed through time. In addition, a single industrial green waste composting process was sampled on a limited number of time points. We aimed to: (1) clarify if the nematode community succession patterns are recurrent for similar but independent processes (2); assess the relation of nematode community changes, i.e. nematode abundances, number of fungal-feeding nematodes, and the F/F+B ratio, with potentially important variables, i.e. temperature, duration of composting and the microbial community, and (3) compare the nematode and microbial community of the experimental farm composts with commercial green waste compost.

6.3 MATERIALS AND METHODS

6.3.1 Composting sites and sampling

The three farm composts (using the CMC-method) were produced simultaneously in open air windrows on a concrete floor at the experimental farm of the Institute for Agricultural and Fisheries Research (ILVO at Merelbeke, Belgium). Three compost piles (50 m long, 3 m wide and 1.5 m high with 3 m^3 feedstock materials per lineal meter) were set up with different ratios of a) grass hay and straw of clover and b) ground poplar bark, i.e. 25/75% (vol/vol), 50/50% (vol/vol) and 75/25% (vol/vol), respectively. The amount of grass hay in the three compost piles was 0%, 23% and 46% (vol/vol), respectively. We will further refer to these composting processes as Farm 1, Farm 2 and Farm 3. Each composting process is monitored individually and management practices are only executed if on a given moment these practices are required for this particular process. In all three composts urea was added at the start to correct the C/N content of the

feedstock towards 30/1, which is generally considered an ideal starting ratio for composting (Zorpas 2009), and cane molasses plus spoiled ensilaged maize were mixed in the feedstock as a compost starter. The heaps were turned on day 3, 10 and on day 83, after which the heap was stored for maturation. The water content was managed by a combination of placing and removing the canvas cover depending on the precipitation and temperature forecast and by manually adding water (2000 L added to Farm 1 on day 10). Samples were taken from the feedstock (day 0) and on day 3, 7, 10, 17, 24, 35, 49, 63, 77, 105, 119, 133, 147, 175, 203. On each of these 16 consecutive sampling events, three composite samples were taken for each of the three compost processes.

The non-experimental industrial green waste compost was produced by the Inter-municipal Society of Public Health in Moen, Belgium. At this site, the integrated processing of household wastes takes place, of which the aerobic composting of green waste materials is an important part. Every year, about 30,000 ton green waste is processed into 'VLACO' labeled compost. This label is provided by the Flemish Compost Organization and guarantees high quality compost with a lot of nutrients, high levels of organic matter and absence of diseases and weeds. In contrast, with the CMC-method the green waste composting uses standardized procedures for all processes with predetermined time schedules for management practices and duration of the process. The used composting method consisted of 5 phases. During the first phase a mixture of available feedstock materials was made (in this case: 35% grass, 15% mixed green waste, 40% wood chips, 10% roots of trees). These materials (± 700 m^3, 450 kg/m^3) were then placed into a long heap (50 m long, 8 m wide and 3 m high) and covered with a membrane for four weeks. Afterwards, the cover was removed, water was added (25,000 L) and the heap was turned mechanically (phase 2). Then the heap was covered again and turned after two weeks (phase 3). After this the heap was kept uncovered and turned with a three-week interval (phase 4) to mature. Finally the compost is sieved and the fraction < 15 mm is sold as compost (phase 5). Sampling took place from the freshly mixed materials (day 0) and during every turning event in each phase (respectively on day 33, 39, 61 and 83). Per sampling event, three composite samples were taken.

Each composite sample consisted of 20 thoroughly mixed randomly chosen samples (50 ml each in the farm processes and 1 L each in the green waste process), and of this total volume (respectively 1 L and 20 L), a subsample of 400 ml was taken for nematode extraction. A mathematical justification of taking a subsample from a bulk sample to estimate nematode population densities is given by Been and Schomaker (2006). A part of the composite samples (±

600 ml) were freeze dried (Christ, Gamma 1-20, Osterode am Harz, Germany), grinded and stored at -4 °C for carbon nitrogen and PLFA analyses.

6.3.2 Abiotic variables

Temperature and CO_2 content of the farm composts were measured at 3 random locations in the heap using specialized equipment (respectively, Digital Thermometer GTH 1150 and Brigon Messtechnik D-63110 Rodgau). Temperature of the green waste compost was continuously measured at a depth of 50 cm using a 5 probe thermocouple (Thermibel, Belgium). At every sampling moment moisture content (%), pH and C/N ratio were measured in three replicates. Extractions of 20 g compost in 100 ml distilled water were shaken by hand three times every two hours and the pH was measured with standard electrodes (Consort P400, Turnhout, Belgium). The moisture content was calculated by determining the dry weight of 50 ml compost after incubation for 48 h at 120 °C. Total C and N contents were measured with a Variomax CNS element analyzer (Elementar GmbH, Hanau, Germany) applying the Dumas method (EN[7] 13654-2).

6.3.3 Nematode community analyses

The nematode communities of all samples (day 0, 3, 7, 10, 17, 24, 35, 49, 63, 77, 105, 119, 133, 147, 175, 203) of the farm composts and all samples (day 0, 33, 39, 61, 83) of the industrial green waste compost were analyzed (see 4.3.3 in Chapter 4 for a detailed description).

6.3.4 Microbial community analyses

Phospholipid fatty acids (PLFAs) were analyzed from all sampling days (except for days 119, 133 and 147) of the farm composts and from all sampling days of the industrial green waste compost (see 4.2.4 in Chapter 4 for a detailed description).

6.3.5 Statistical analysis

Nematode density changes during the compost processes were analyzed using one-way ANOVA on double square root transformed data and subsequent post hoc Tukey HSD tests in Statistica 6.0 (Statsoft Inc.). Nematode community compositions during the farm processes were compared using non-metric Multi-Dimensional Scaling (nMDS) based on Bray-Curtis similarity matrices of square-root transformed abundance data (numbers/100 g dry weight compost) using Primer 6 (Clarke and Warwick 2001). A stepwise BIOENV procedure was run in Primer 6 to

[7] EN: European Standard. European Standards are developed by CEN, the European Commission for Standardization. Numbers refer to the specific protocols.

select the best explaining abiotic variables (duration of composting, C/N ratio, pH, moisture content, CO_2).

Total PLFA concentrations and the relative concentrations of the functional groups of the green waste compost (i.e. Gram-positive, Gram-negative, Fungi, total bacteria, AMF and Actinomycetes) were compared between sampling days using one-way ANOVA and subsequent post hoc Tukey HSD tests in Statistica 6.0. Changes in the concentrations of the marker fatty acids (mol%) in the farm composts were analyzed using Principal Component Analysis (PCA) in Primer 6. Since assumptions for one-way ANOVA of normality and homogeneity of variances could not be fulfilled, the functional group concentrations of Gram-positive and Gram-negative bacteria, fungi, AMF (Arbuscular Mycorrhizal Fungi) and Actinomycetes of the different composting phases (based on the shifts in the nematode community, indicative delineation of composting phases was performed, see 6.4.2) were compared using non-parametric Kruskal-Wallis and Mann-Whitney U pairwise tests in Statistica 6.0. with Bonferroni corrections (α= 0.013).

The relationship between the explanatory variables and the nematode abundance or the absolute number of fungal-feeding nematodes or the proportion of fungal-feeding nematodes to the total number of fungal- plus bacterial-feeding nematodes was modeled by means of a generalized linear mixed model with a normal, Poisson and binomial distributed error, respectively. The explanatory variables were temperature, the amount of fungal PLFA, day and a quadratic day effect to account for a possible non-linear relationship between the dependent variable and time. Given that the time intervals at which nematode numbers were assessed were shorter at the beginning of the experiment, variable day was log transformed before analysis. As nematode densities were measured repeatedly on each of the three farm compost heaps, we also accounted for temporal autocorrelation in all models by fitting a first order autoregressive variance structure to the residual errors. Compost heap was further included as a random effect. Degrees of freedom were adjusted by means of the Kenward-Roger approximation. Models with the absolute number of fungal- feeding nematodes or the proportion of fungal-feeding nematodes to the total number of fungal- plus bacterial- feeding response variables were fitted by means of the GLIMMIX procedure and the model with nematode abundance as response variable was fitted to the log transformed values by means of the MIXED procedure in SAS v.9.3. (SAS Institute Inc., Cary, NC, USA).

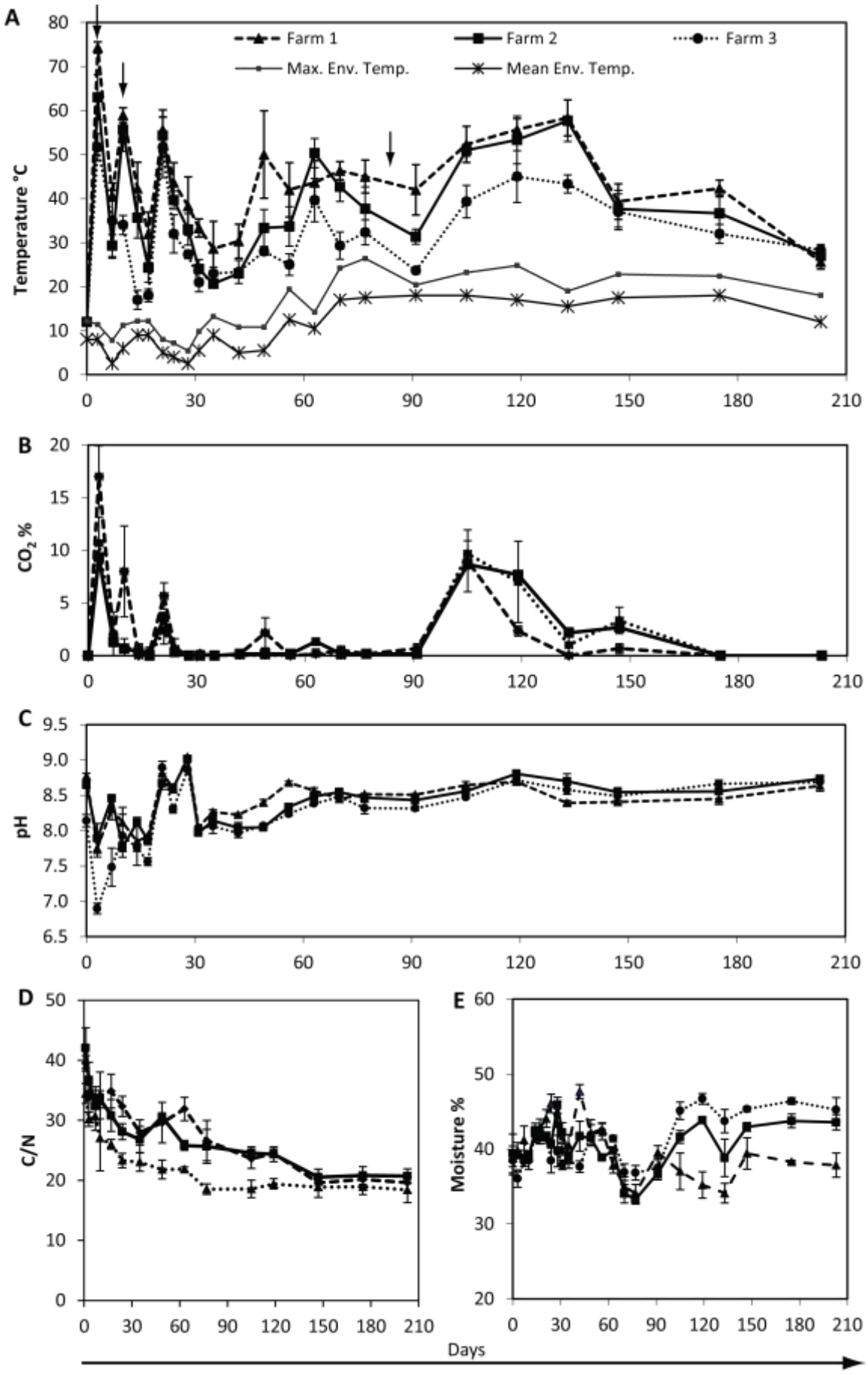
6.4 RESULTS

6.4.1 Abiotic variables

The abiotic variables of Farm 1, Farm 2 and Farm 3 exhibited very similar trends with time (Figure 6.1). Given the considerable temperature fluctuations the compost process cannot be categorized in the typical temperature related compost phases, i.e. thermophilic, cooling and maturation, as described in Chapters 2 and 4. However, since temperature did not simply decrease with time, a differentiation between the temperature and the time effect on the nematode community was still possible (see 6.2.5). The processes started with characteristic heat peaks where temperatures reached up to 75 ± 1.3 °C in Farm 1, 63 ± 5.1 °C in Farm 2 and 52 ± 3.1 in Farm 3 (Figure 6.1A). The turning of the compost heaps on day 10 caused a temporary temperature drop (arrow in Figure 6.1A). Afterwards, from day 24 - 62, the temperatures decreased (< 40 °C), increased (up to 50, 35 and 30 °C in farm 1, 2 and 3 respectively) and decreased again. From day 91 a distinct gradual increase of temperatures was observed, with maxima on day 119 - 133. These maxima (i.e. 58 ± 4.1, 58 ± 4.7 and 43 ± 2.1 °C in Farm 1-3, respectively) were only slightly lower than the maxima during the primary heat peaks. The increased temperatures coincided with a period of increased carbon dioxide (CO_2) production (Figure 6.1B). From day 133 - 203 the temperatures gradually decreased to near ambient levels. The pH fluctuated during the first month with maximum levels (8.8-9) on day 28. Afterwards the pH first decreased and then increased again to remain more or less constant (Figure 6.1C). The C/N ratio gradually decreased with time from 35-40 to approximately 20 on day 105 and afterwards remained stable (Figure 6.1D). The moisture content was always higher than 30% but never exceeded 47% (Figure 6.1E).

In the green waste compost process no heat peaks could be distinguished because temperatures were always very high (between 50 and 80 °C), except for the short periods of time after the heap was turned when temperatures reached more or less ambient levels (arrows in Figure 6.2A). The pH increased from 5.3 ± 0.24 to levels between 6.7 ± 0.36 and 7.8 ± 0.11 (Figure 6.2B). The C/N ratio gradually decreased from 22.5 ± 0.27 to 13.1 ± 0.56 (Figure 6.2C) and the moisture content hardly exceeded 10% except for day 33 when water was added and the moisture content increased to 30%.

Figure 6.1: Abiotic variables measured during the three farm composting processes (Farm 1, Farm 2 and Farm 3) including: (A) Temperature of the compost (°C) and mean and maximum ambient temperatures (°C) per day (24h), (B) CO_2 content (%), (C) pH values, (D) C/N ratio, (E) moisture content (%). Error bars indicate SD based on three replicates. Arrows indicate turning events of the heaps.


A
Farm 1
Farm 2
Farm 3
Max. Env. Temp.
Mean Env. Temp.
Temperature °C
B
CO_2 %
C
pH
D
C/N
E
Moisture %
Days

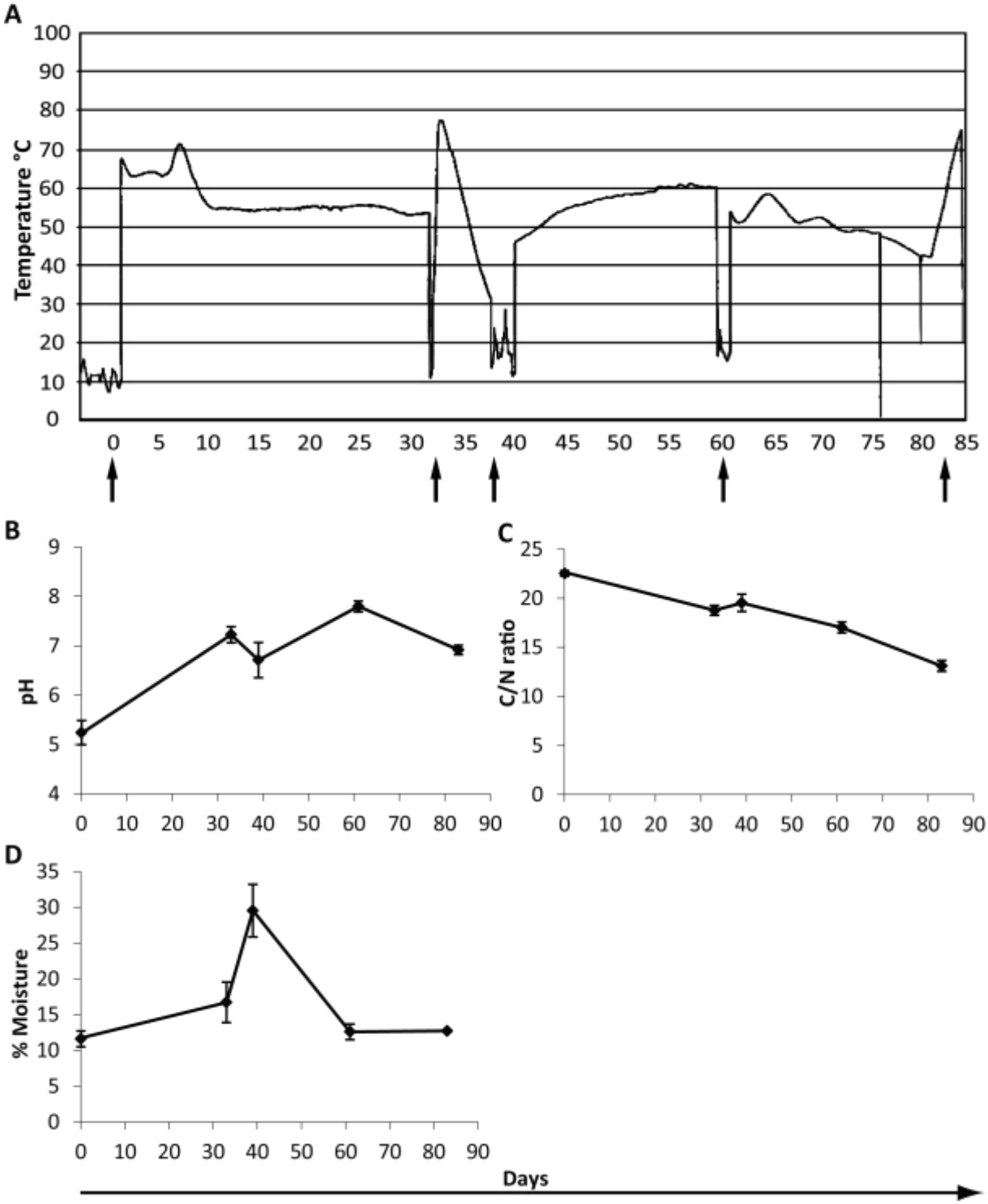


Figure 6.2: Abiotic variables measured during the green waste composting processes including: (A) Temperature of the compost (°C), arrows indicating turning and sampling events, (B) pH values, (C) C/N ratio, (D) moisture content (%). Error bars indicate SD based on three replicates.

6.4.2 Nematode community

In the farm compost processes nematode numbers sharply increased on day 17, shortly after turning the heaps when the temperature dropped (i.e. 7920 ± 1603, 3792 ± 1137 and 9575 ± 2407 nematodes/100 g DW compost in Farm 1, Farm 2 and Farm 3, respectively) (Figure 6.3 and Table 6.1). Also in the continuation of the process the nematode densities were virtually inversely related with temperature: when the latter increased the nematode densities decreased and *vice versa*. Around 40 °C appeared to be the threshold for nematode densities to decrease or increase. For nearly all sampling moments above 40 °C, nematode densisties were significantly lower except for day 7 where both low nematode abundance and low temperature were measured. The nematode abundances were significantly negatively related with temperature ($p<0.001$) but not with duration of composting (day) (MIXED procedure in SAS).

Thirty-one genera belonging to 14 families were identified from the farm processes (see also Supplementary Tables 6.S1, 6.S2, 6.S3), the most common taxa being *Aphelenchoides* sp., *Mononchoides composticola*, *Poikilolaimus* sp., *Pelodera* sp. and *Halicephalobus* cfr. *gingivalis*. Remarkably similar patterns were present in the three farm processes (Farm 1: Figure 6.4A, Farm 2: Figure 6.5A, Farm 3: Figure 6.6A). During days 0 - 10, the bacterial-feeding enrichment opportunists (cp-1) dominated the nematode community. The most prominent species in this phase were *Rhabditella axei*, *Pelodera teres*, *Pelodera cylindrica* and *Poikilolaimus* sp., including a high number of Rhabditidae that were found in dauer phase. Afterwards, from day 17 - 49, the bacterial-feeding/predators (*Mononchoides composticola*) became dominant, although this dominance was less pronounced for Farm 3, especially for day 24 where their proportion accounted only for 7 ± 4.1%. The proportion of fungal-feeding nematodes, especially *Aphelenchoides* sp., increased from day 49 but decreased again with persistent high temperatures. Around day 119 the nematode community in farm 1 and 2 again changed towards a dominance of bacterial-feeding enrichment opportunists (mainly *Poikilolaimus* sp.). From day 147 to day 203 the relative share of the fungal-feeding nematodes increased again, including other taxa than *Aphelenchoides* sp., such as *Ditylenchus* sp., Neotylenchidae spp. and Tylenchidae spp. (cp-2). Other general opportunists, such as *Eucephalobus* sp. (bacterial-feeding) and *Seinura* sp. (predator)(in Farm 1 and 2), also became numerically more important during this phase. The F/F+B ratio (Figure 6.4B, 6.5B and 6.6B; Table 6.1) and the MI (Table 6.2) index values generally increased during the process but with considerable fluctuations.

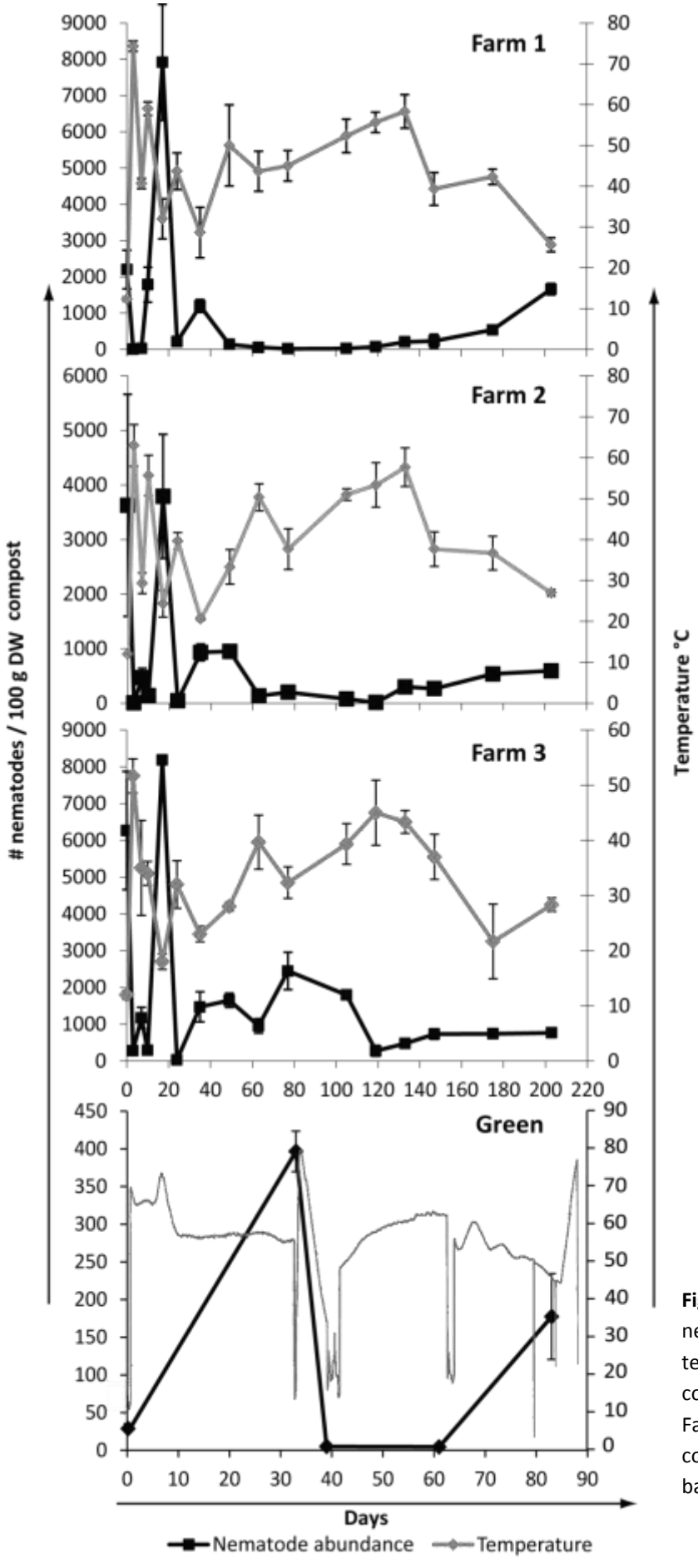


Figure 6.3: Nematode abundances (# nematodes/100 g DW compost) and temperature (°C) during the three farm composting processes (Farm 1, Farm 2 and Farm 3) and during the green waste composting (Green). Error bars indicate SD based on three replicates.

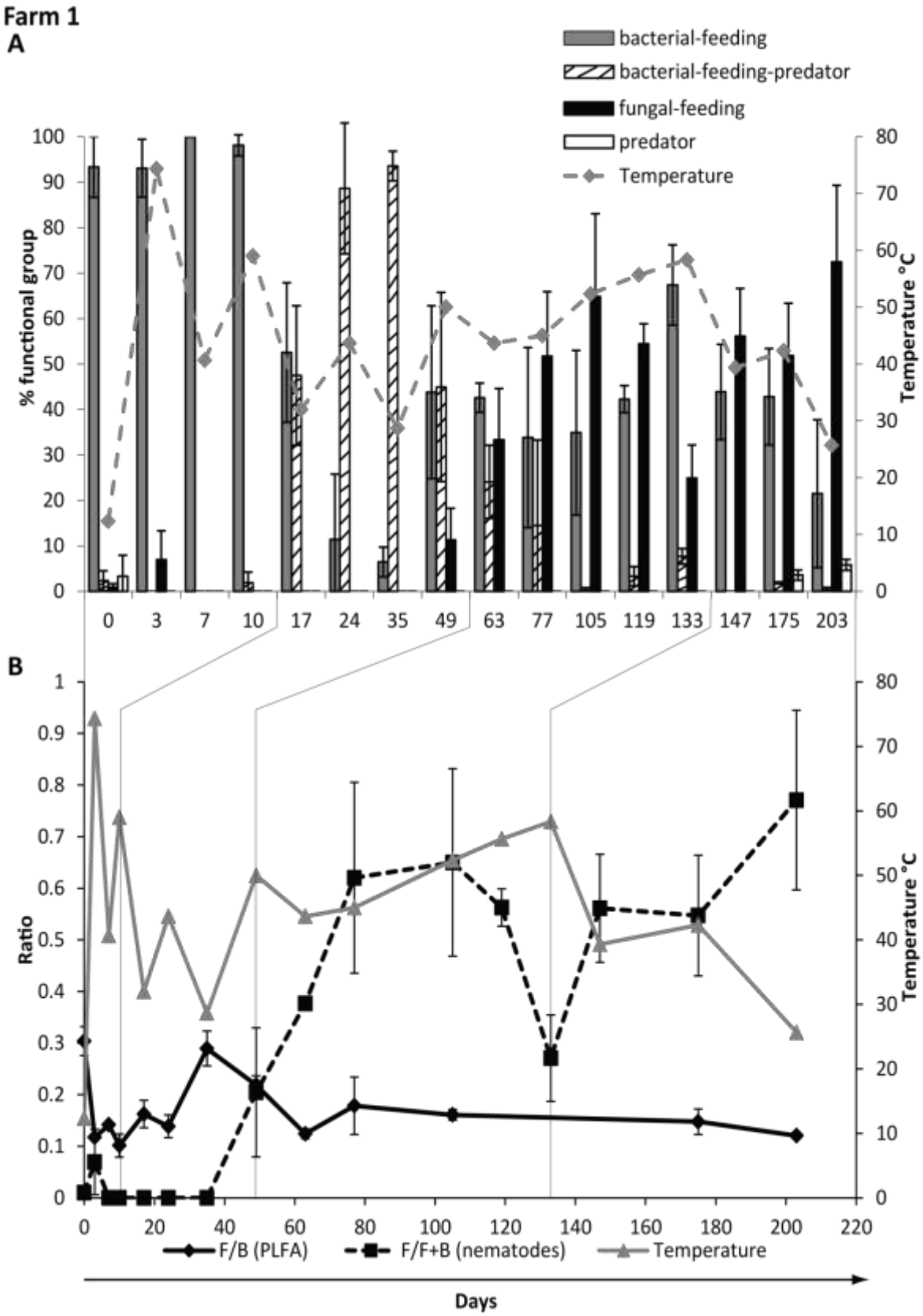


Figure 6.4: Farm 1. (A) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator at every sampling moment. Omnivores (low in abundance) are not represented in the graph. (B) F/B ratio based on fungal and bacterial PLFAs and F/F+B ratio based on the fungal- and bacterial-feeding nematode densities. Standard deviations are indicated as error bars. Vertical lines represent phases which were indicatively delineated based on key-point changes in the nematode community.

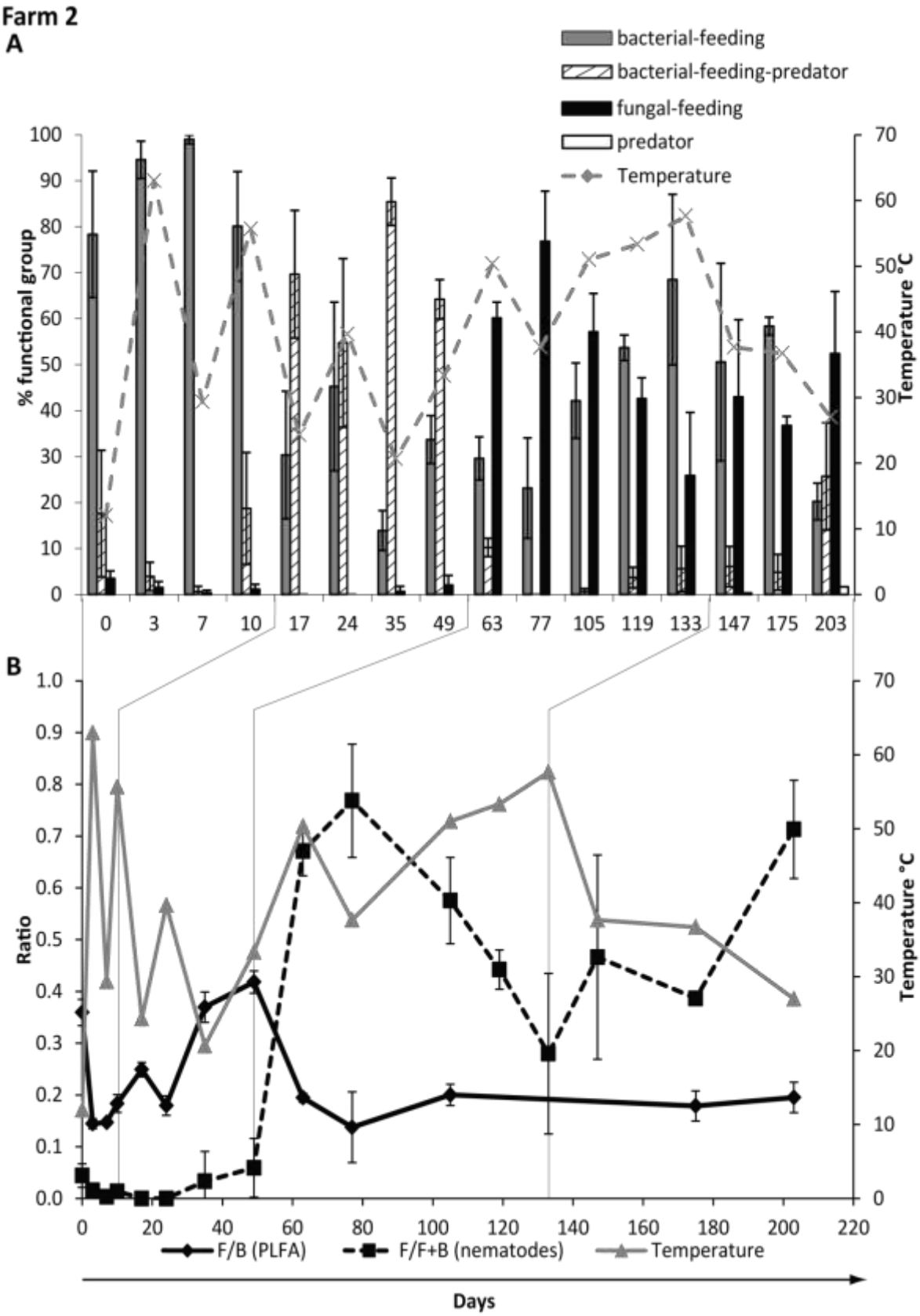


Figure 6.5: Farm 2. (A) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator at every sampling moment. Omnivores (low in abundance) are not represented in the graph. (B) F/B ratio based on fungal and bacterial PLFAs and F/F+B ratio based on the fungal- and bacterial-feeding nematode densities. Standard deviations are indicated as error bars. Vertical lines represent phases which were indicatively delineated based on key-point changes in the nematode community.

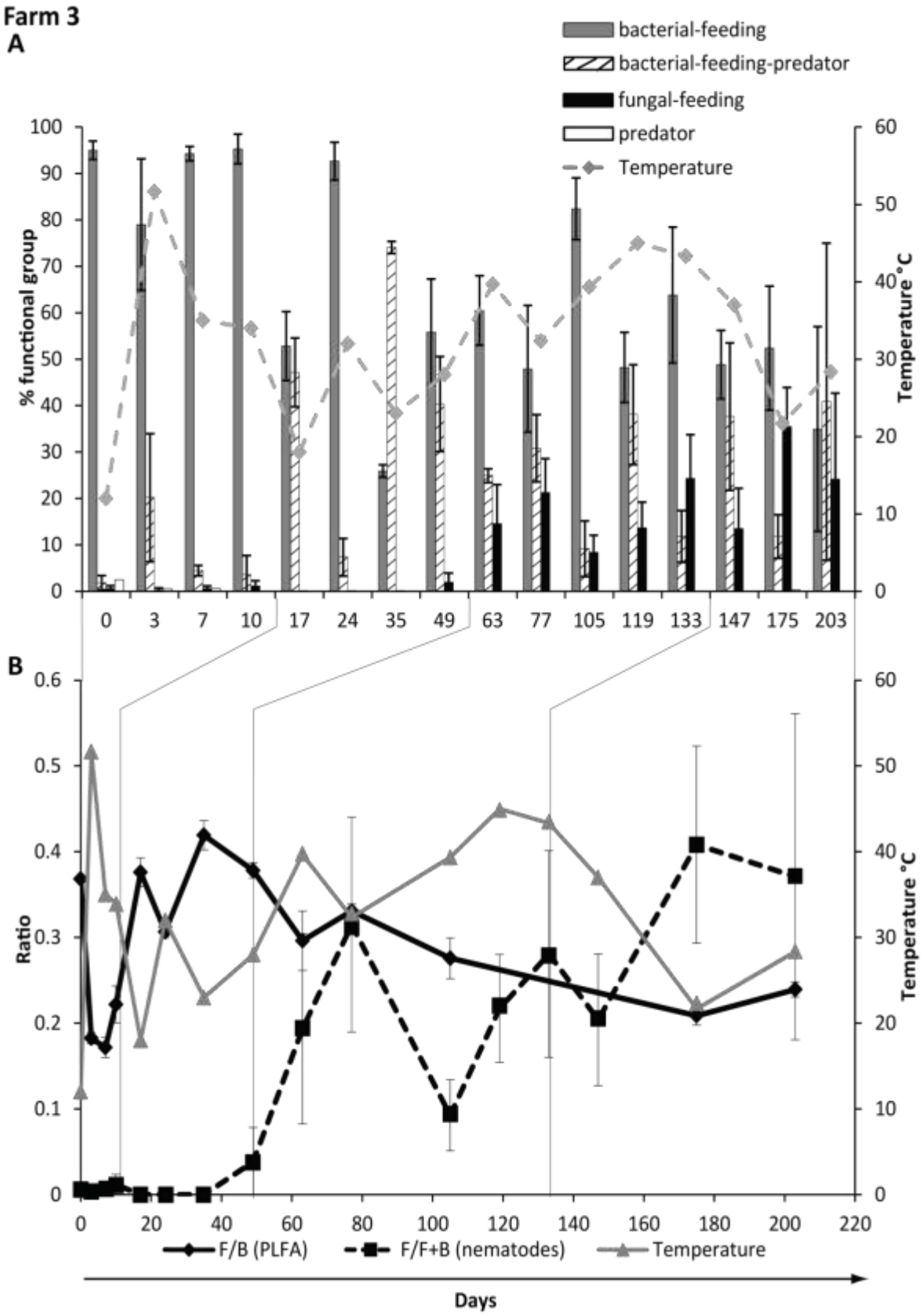


Figure 6.6: Farm 3. (A) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator at every sampling moment. Omnivores (low in abundance) are not represented in the graph. (B) F/B ratio based on fungal and bacterial PLFAs and F/F+B ratio based on the fungal- and bacterial-feeding nematode. Standard deviations are indicated as error bars. Vertical lines represent phases which were indicatively delineated based on key-point changes in the nematode community.

The absolute number of fungal-feeding nematodes was significantly negatively related with temperature (p=0.001) and positively with duration of composting (day) (quadratic effect of time, GLIMMIX procedure in SAS, p=0.029). Plotting the predicted against the observed number of fungal-feeding nematodes revealed that both variables explained the number of fungal-feeding nematodes fairly well (Figure 6.7A). In the samples dominated by the bacterial-feeding/predators (day 17-35), none or very few fungal-feeding nematodes were found while the model predicted higher numbers. The ratio F/F+B was also strongly positively related with duration of composting (quadratic day effect p<0.001, day effect p=0.029) but not with temperature (GLIMMIX procedure in SAS). Figure 6.7B displays the observed F/F+B ratio against the predicted F/F+B ratio based on the estimated model parameters.

The nMDS of the farm composts clearly confirms that the nematode communities were primarily grouped according to duration of composting rather than to the compost process (i.e. Farm 1, Farm 2 and Farm 3) they originated from (Figure 6.8). The species composition in the compost was best explained by a combination of duration of composting and C/N ratio (r= 0.53, stepwise BIOENV).

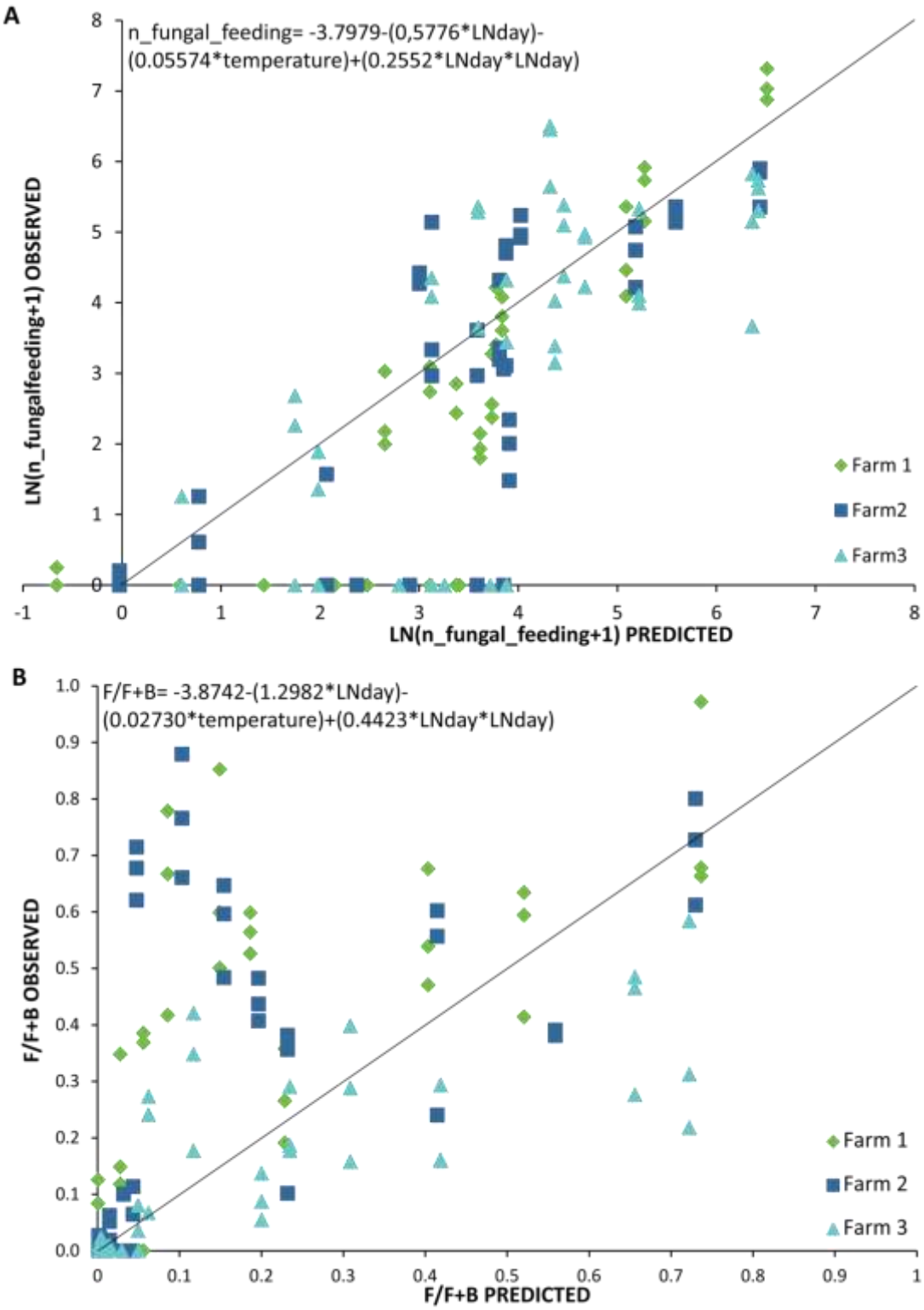


Figure 6.7: (A) The predicted against the observed numbers of fungal-feeding nematodes for Farm 1, 2 and 3. (B) The predicted against the observed F/F+B ratio for Farm 1, 2 and 3.

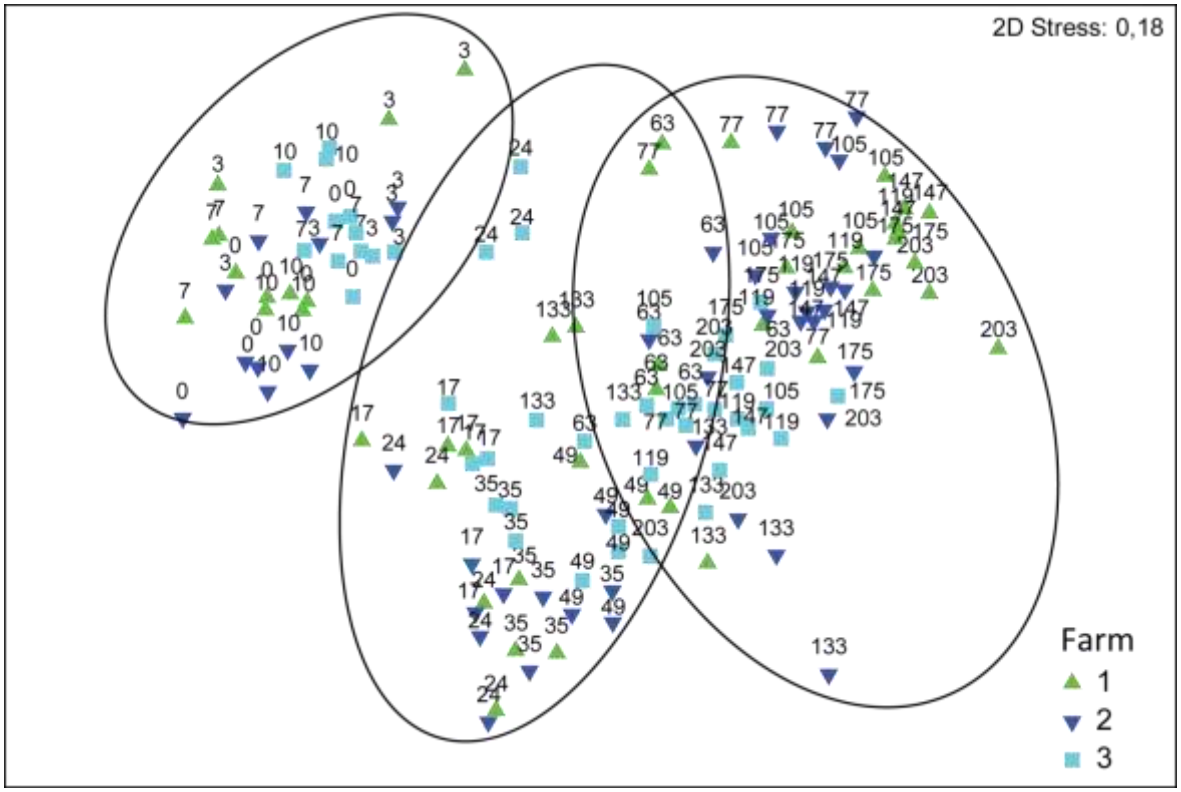


Figure 6.8: MDS ordination of square root transformed nematode species abundances of every sampling moment of the three farm composts. Duration of composting (days) is indicated as a number next to the mark of the sample.

Compared to the farm processes the nematode densities and the nematode community structure of the green waste process were completely different. Nematode densities were very low, from 3 ± 5 nematodes/100 g DW to maximum 400 nematodes /100 g DW (Figure 6.3 and Table 6.2), compared to up to 9575 ± 2407 nematodes /100 g DW in farm compost (Figure 6.3 and Table 6.1). Nematodes were even absent in 2 replicas of day 39 and 1 replica on day 61. Especially at the end of the compost process there was a pronounced difference in nematode numbers, with only 177 ± 57 in the green waste compost compared to 1008 ± 572 in the farm composts. Only 13 genera belonging to 7 families were detected (see also Supplementary Table 6.S4) and all taxa found in the green waste process were also found in the farm processes, except for *Procephalobus* sp. which was pre-eminently the most common genus in the industrial green waste compost (64% of the nematode community). The bacterial-feeding enrichment opportunists, such as *Procephalobus* sp., were omnipresent and made up more than 80% of the community in all samples (except for day 61) (Figure 6.9A). Of the fungal-feeding nematodes, only *Aphelenchoides* sp. was found; it was present on all days, except for day 39, but never reached proportions higher than 8%. The bacterial-feeding/predator *Mononchoides composticola* occurred solely on day 33 and made up only 7% of the community. In contrast with the farm processes, the nematode based indices (F/F+B and MI) hardly changed during the

green waste process. The F/F+B ratio was more or less zero (Figure 6.9B) while the MI was constantly close to one (Table 6.2).

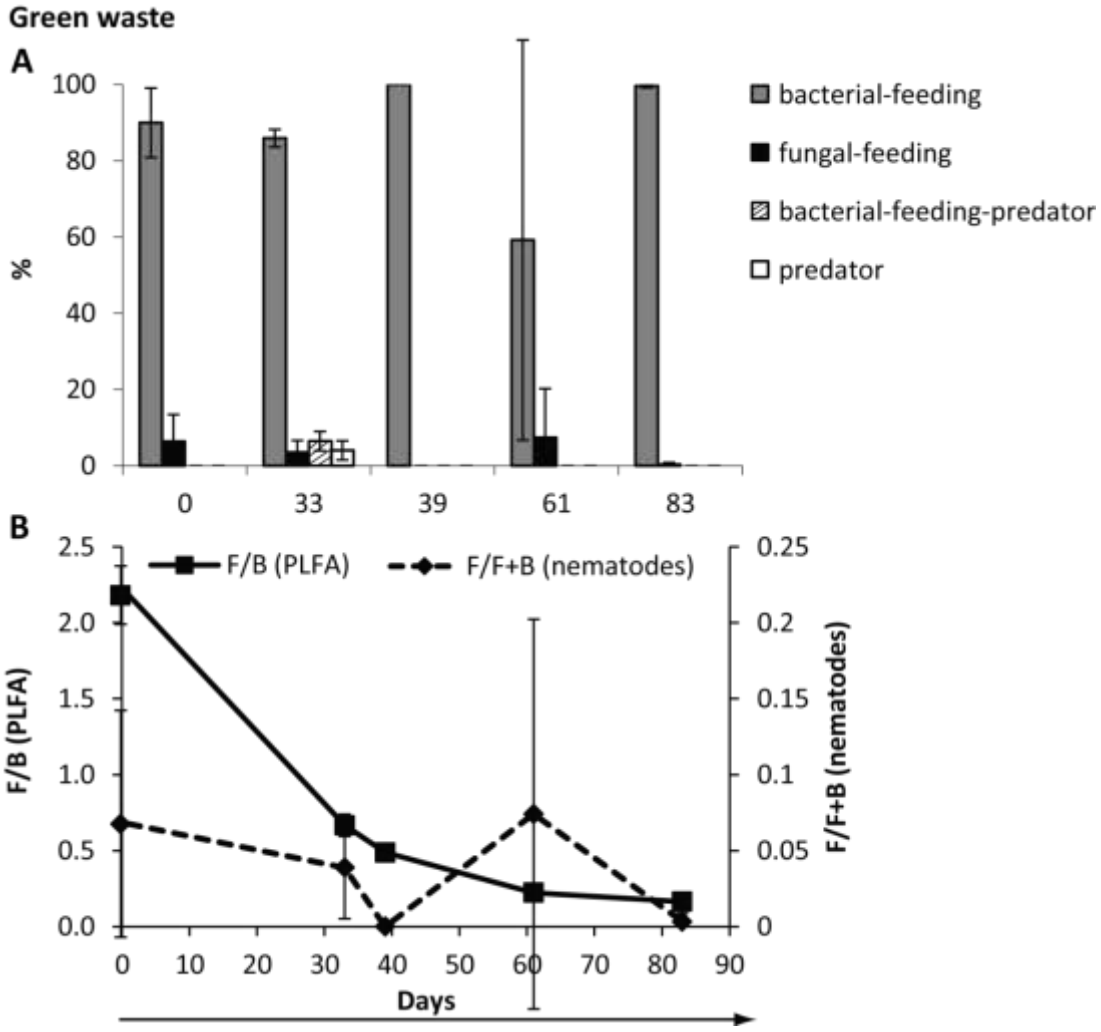


Figure 6.9: (A) The percent contribution of each feeding type (fungal-feeding, bacterial-feeding, bacterial-feeding/predator at every sampling moment of the green waste process. Omnivores are not represented in the graph because they only occurred in one sample. (B) F/B ratio based on fungal and bacterial PLFAs and F/F+B ratio based on the fungal- and bacterial-feeding nematode densities during the green waste process. Standard deviations are indicated as error bars based on three replicates.

Table 6.1: Overview of the means ± SD (based on three replicates) of the abundance of nematodes (per 100g dry weight compost), number of genera and the values of the indices: F/F+B (fungivorous/ (fungivorous+bacterivorous) ratio), MI (Maturity Index) at every sampling moment during the farm processes.

Day		Abundance	# Genera	F/F+B	MI		Abundance	# Genera	F/F+B	MI		Abundance	# Genera	F/F+B	MI
0		2203±531	9±2	0±0.01	1.1±0.07		3628±2034	8±1	0±0.02	1.2±0.06		6266±1604	9±1	0±0.01	1±0
3		6±5	3±1	0.1±0.06	1.1±0.06		10±2	9±2	0±0.01	1±0.01		282±27	12±3	0±0.01	1±0.02
7		28±3	4±1	0±0	1±0		469±170	5±2	0±0.01	1±0.02		1170±287	13±1	0±0.01	1±0.01
10		1784±485	5±1	0±0	1±0		141±41	6±1	0±0.01	1±0.01		292±52	11±2	0±0.01	1±0.01
17		7920±1603	7±2	0±0	1±0		3792±1137	4±0	0±0	1±0		9575±2407	8±1	0±0	1±0.01
24		214±114	4±2	0±0	1±0		47±10	4±2	0±0	1±0		33±20	5±2	0±0	1±0
35		1199±176	4±1	0±0	1±0		933±153	4±2	0±0.06	1±0.01		1470±408	5±1	0±0	1±0
49	*Farm 1*	140±35	8±3	0.2±0.13	1.1±0.07	*Farm 2*	951±78	7±3	0.1±0.06	1±0.02	*Farm 3*	1650±193	6±2	0±0.04	1.1±0.01
63		54±14	5±5	0.4±0.01	1.3±0.01		137±20	6±1	0.7±0.05	1.6±0.03		957±195	7±1	0.2±0.11	1.1±0.08
77		17±3	5±1	0.6±0.18	1.5±0.17		203±19	4±1	0.8±0.11	1.9±0.01		2446±509	6±0	0.3±0.13	1.2±0.07
105		26±16	6±1	0.7±0.18	1.8±0.15		80±67	5±1	0.6±0.08	1.7±0.13		1797±130	7±1	0.1±0.04	1.1±0.04
119		73±34	8±1	0.6±0.04	1.7±0.05		15±8	8±1	0.4±0.04	1.5±0.03		278±144	6±1	0.2±0.06	1.1±0.05
133		206±80	10±1	0.3±0.08	1.2±0.07		302±81	6±2	0.3±0.15	1.3±0.14		479±71	6±2	0.3±0.12	1.2±0.09
147		229±191	6±1	0.6±0.1	1.9±0.01		268±53	8±2	0.5±0.2	1.7±0.04		734±138	7±2	0.2±0.08	1.2±0.1
175		537±80	9±0	0.5±0.12	1.8±0.06		538±68	8±0	0.4±0	1.5±0.1		740±45	8±2	0.4±0.12	1.4±0.08
203		1662±157	7±1	0.8±0.17	1.8±0.15		596±52	9±1	0.7±0.09	1.5±0.15		768±113	8±1	0.4±0.19	1.3±0.2

Table 6.2: Nematode community during green waste composting with means ± SD based on three replicates of the abundance of nematodes (per 100g dry weight compost), number of genera and the values of the indices: F/F+B (fungivorous/ (fungivorous+bacterivorous) ratio), MI (Maturity Index) during the green waste process. Microbial community during green waste composting with means ± SD based on three replicates of total PLFA (nmol/g dry soil), F/B ratio and main biomarker concentrations (mol%).

	DAY				
	1	**33**	**39**	**61**	**83**
Nematode community					
Abundance	34 ± 1.4	397 ± 27	3 ± 5.5	4 ± 4.12	177 ± 56.93
F/F+B	0.07 ± 0.07	0.04 ± 0.03	0	0.07 ± 0.13	0.003 ± 0.01
MI	1.2 ± 0.14	1.1 ± 0.06	1	0.8 ± 0.65	1 ± 0.01
Microbial community					
Gram +	16.7 ± 1.10	38.9 ± 1.67	46.5 ± 0.79	59.1 ± 2.31	62.3 ± 0.49
Gram -	11.3 ± 0.74	15.5 ± 1.97	13.6 ± 0.71	12.7 ± 0.36	13.7 ± 0.19
Actinomycetes	0.8 ± 0.02	2.2 ± 0.11	2.4 ± 0.20	3.3 ± 0.36	3.2 ± 0.04
Total Bacterial	31.2 ± 1.90	58.7 ± 2.51	65.6 ± 1.47	79 ±2.37	83.2 ± 0.44
Fungi	67.9 ± 1.92	39.1 ± 2.51	32.0 ± 1.65	17.6 ± 2.02	13.7 ± 0.42
Total PLFA	1963 ± 125	1565 ± 153	822 ± 108	995 ± 34	995 ± 51
F/B	2.2 ± 0.19	0.7 ± 0.07	0.5 ± 0.04	0.2 ± 0.03	0.2 ± 0.01

6.4.3 Microbial community

According to the PLFA data, the microbial community of the farm composts, changed mainly during the first weeks of composting while afterwards the PLFA based patterns were remarkably stable (Table 6.3). This is confirmed by the PCA of the relative biomarker concentrations which resulted in a clear discrimination, mainly along the first axis, between early (until day 17) and later compost stages (Figure 6.10A) independent of the compost process (i.e. Farm 1, Farm 2 and Farm 3). The increasing 10Me16:0 (Actinomycetes) and decreasing 18:1ω7 (Gram-negative) biomarkers contributed most to the first axis (explains 56% of the variation), while the second axis (26% of the variation) was defined most importantly by decreasing fungal and AMF (18:2ω6,9 and 16:1ω5C respectively) biomarkers and the increasing Gram-negative biomarker i16:0 (Figure 6.10B).

The total PLFA was highest after the heat peaks on day 35 and/or day 49 and afterwards decreased again (Table 6.3). Except for day 0 in all farm processes and day 7 and 17 in Farm 3, all samples showed a distinct dominance of Gram-positive bacteria. Comparison of the relative contributions of the functional groups to the microbial community between indicatively delineated composting phases based on the changes in the nematode community (i.e. day 0-10, day 17-49, day 63-133, day 147-203) (see 6.4.2) revealed similar differences in all farm processes mainly between the first phase (day 0-10) and the other phases. The Gram-negative bacteria

were highest during the first 10 days of composting and their concentrations were significantly lower during days 17-49 and days 63-133 compared to days 0-10. The Actinomycetes gradually increased during the process, which is confirmed by significantly higher concentrations during days 63-133 and days 147-203 compared to days 0-10. The fungal PLFA decreased in the beginning of the process as temperatures increased but peaked on day 35 in Farm 1 and on day 35 and day 49 in Farm 2 and 3, and afterwards decreased again with significantly lower concentrations during days 147-203 compared to days 17-49. In accordance, the F/B ratio initially decreased with increasing temperatures and peaked on days 35-49, with significantly higher values on day 35 (Farm 1 & 2; $p<0.04$, <0.001) and day 49 (Farm 3 $p<0.05$) compared to all later stages, except between day 49 and 77 in Farm 3. Unlike the steadily increasing proportions of fungal-feeding nematodes, the fungal PLFA remained remarkably stable and relatively low after day 63 even when temperatures reached near ambient levels. The amount of fungal PLFA had no significant effect ($p\geq0.05$) in the models (GLIMMIX procedure in SAS) of the absolute number of fungal-feeding nematodes and thus no additional information was explained by adding this variable in the model.

In the green waste compost process, the total PLFA on day 0 and day 33 was significantly higher ($p<0.001$) compared to days 39, 61 and 83. During the process, the fungal PLFA significantly ($p<0.001$ between all samples) decreased with time, while the share of the Gram-positive bacteria increased with time ($p<0.03$ between all samples). On day 0 the most contributing fungal PLFA was 18:1ω9, but afterwards on day 39 18:1ω9 and 18:2ω6,9 contributed equally. The most prominent Gram-positive biomarker was i16:0 and for the Gram-negative bacteria first (day 0 and 33) both 16:1ω7 and 18:1ω7 and later (day 39, 61, 83) cy17:0 and cy19:0 were dominant. According to the decreasing fungal PLFA, the F/B ratio also sharply decreased from 2.2 ± 0.19 on day 0 to 0.2 ± 0.01 on day 83 (Figure 6.10B).

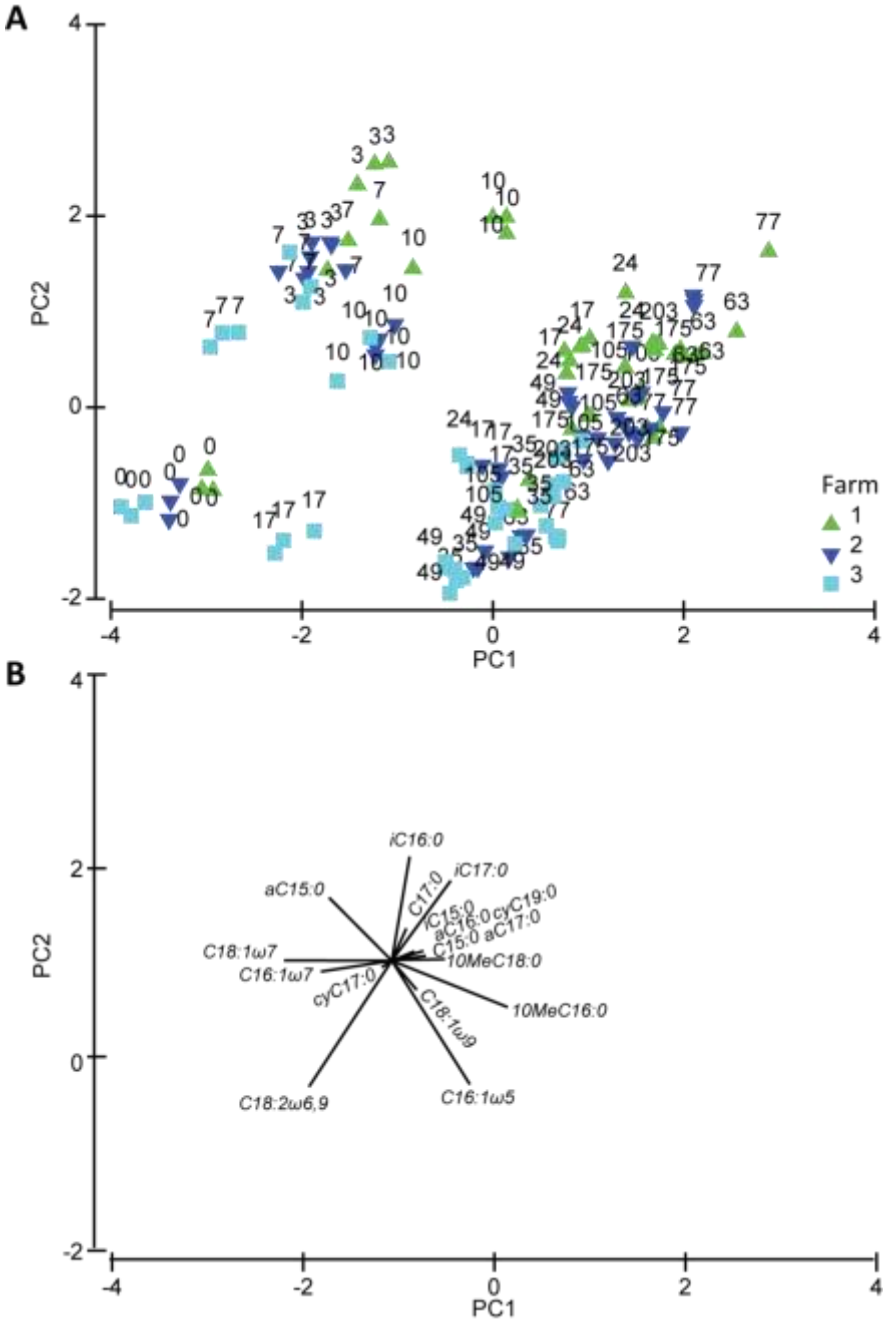


Figure 6.10: (A) Two-dimensional PCA ordination of biomarker fatty acids (mol%) of the three farm composting processes (Farm 1, Farm 2, Farm 3). (B) Vector loading plot with all individual marker fatty acids.

Table 6.3: Total PLFA (nmol/g dry soil), F/B ratio and main biomarker concentrations (mol%) ± standard deviation, during the farm processes. * The used biomarker for AMF is questionable in a compost environment (see 4.3.4).For completeness the concentrations of this group are provided but not used in further community analyses

Day	Gram +	Gram -	Total Bact	Actinomycetes	Fungi	AMF*	Total PLFA	F/B
				Farm 1				
0	30±0.69	40.1±0.88	72.6±1.58	2.1±0.05	22±1.57	3.31±0.05	1287±95.9	0.3±0.03
3	61.5±1.79	22.9±1.38	87.9±0.43	1.1±0.01	10.3±0.39	0.63±0.06	2103±152.6	0.1±0.01
7	53.2±3.95	28.6±3.8	85±0.55	1.4±0.08	12±0.38	1.58±0.25	1767±238.4	0.1±0.01
10	55.4±0.86	21.5±2.01	83.4±0.42	5.4±2.09	8.5±1.89	2.77±0.19	1921±250.7	0.1±0.02
17	51.7±1.95	17.7±1.01	73.2±2.11	7.1±0.13	11.8±1.58	7.92±0.42	1724±110.4	0.2±0.03
24	54±2.98	16.8±1.98	75±1.62	6.7±0.74	10.4±1.48	7.86±1.53	1674±527.6	0.1±0.02
35	42.1±1.64	20±0.26	64.4±1.9	7.6±0.16	18.6±1.67	9.31±0.23	3182±126.9	0.3±0.03
49	47.4±0.57	18±0.5	69±0.74	8.3±0.15	15.1±1.04	7.6±0.32	2452±112.1	0.2±0.02
63	50.6±1.63	15.9±2.7	70.5±0.87	13.5±0.68	8.8±0.64	7.3±1.23	1906±173.4	0.1±0.01
77	48.9±8.68	15.3±4.61	68.1±4.65	10.6±0.85	12±3.19	9.32±2.29	2208±337.4	0.2±0.06
105	47.5±0.58	19.1±0.32	70.1±0.61	10.1±0.2	11.3±0.62	8.47±0.1	2204±158.1	0.2±0.01
175	49.4±3.18	17.9±3.88	70.5±2.05	11.2±0.63	10.4±1.48	7.87±1.24	1667±231.7	0.1±0.02
203	49.1±0.4	20.2±0.32	72.3±0.29	11.8±0.21	8.7±0.42	7.15±0.19	1985±140	0.1±0.01
				Farm 2				
0	27.9±1.49	39.7±0.56	70.2±1.4	1.7±0.03	25.2±1.28	2.88±0.12	1297±60.7	0.4±0.03
3	52.5±1.19	29.1±0.92	85.1±0.71	1.5±0.17	12.3±0.71	1.17±0.12	1811±310.2	0.1±0.01
7	49.8±1.73	31.8±1.74	84.6±0.62	1.5±0.19	12.4±0.43	1.47±0.17	2056±199.2	0.1±0.01
10	46.6±1.24	28.9±0.6	78.7±1.16	3.2±0.13	14.4±1.14	3.6±0.15	2136±286.1	0.2±0.02
17	39.9±1.07	22.8±0.86	65.8±1.85	6±0.23	16.4±0.44	11.83±1.19	2342±133.7	0.2±0.01
24	50.9±2.75	17.7±2.65	72.2±0.74	7.8±0.06	13±1.26	7.02±0.64	2841±259.4	0.2±0.02
35	38.1±0.69	19.2±0.33	59.7±0.94	7.1±0.25	22±1.42	11.16±0.72	3581±86.4	0.4±0.03
49	36.8±0.71	20±0.29	59.4±0.89	6.2±0.27	24.8±0.92	9.62±0.25	3128±257	0.4±0.02
63	46.9±1.27	16.3±1.89	66.8±0.59	9.6±0.32	13±0.69	10.52±1.46	2641±346.1	0.2±0.01
77	51.4±3.94	15.5±2.07	71.3±5.99	12.3±2	9.5±3.64	6.96±4.35	1855±296.6	0.1±0.07
105	44.2±1.23	21.4±1.25	68.8±1.29	8±0.46	13.8±1.2	9.34±0.45	2494±751.5	0.2±0.02
175	45.2±0.61	18.4±2.88	67.1±2.88	12.2±0.84	11.9±1.44	8.81±2.44	2105±227.6	0.2±0.03
203	41.9±2.13	20.7±0.48	65.6±1.61	12.4±0.35	12.8±1.61	9.24±0.33	2265±17.1	0.2±0.03
				Farm 3				
0	26.2±0.29	41.9±0.15	70.4±0.23	1.2±0.06	25.9±0.21	2.48±0.13	2055±178.8	0.4±0
3	52.6±1.78	26.8±1.2	82.5±0.58	1±0.18	15.1±0.5	1.47±0.71	2698±84	0.2±0.01
7	39.7±0.27	40.6±0.97	83±0.7	1.1±0.11	14.2±0.84	1.73±0.04	2986±707.1	0.2±0.01
10	43.7±2.41	29.6±1.43	76.8±1.41	2.9±0.18	17±1.35	3.25±0.53	2625±137.7	0.2±0.02
17	30.5±0.95	32.1±0.37	65.1±0.66	2.5±0.18	24.5±0.85	7.88±0.07	3108±487.9	0.4±0.02
24	44.1±0.38	21.1±0.74	68.1±0.77	5.4±0.31	20.9±0.26	5.68±0.3	4123±66	0.3±0.01
35	35.5±0.24	20.8±0.48	58.6±0.59	5.3±0.33	24.5±0.76	11.57±0.31	4773±324.1	0.4±0.02
49	37.5±0.42	21.7±0.49	61.7±0.6	4.8±0.23	23.3±0.46	10.27±0.6	4689±68.9	0.4±0.01
63	39.8±1.72	20.3±0.59	62.8±1.82	6.9±0.53	18.6±1.69	11.81±0.21	3713±171.5	0.3±0.03
77	38.4±0.4	18.7±0.3	59.9±0.15	7±0.18	19.8±0.43	13.31±0.45	3107±75.1	0.3±0.01
105	39.5±0.8	23.2±0.4	65.7±1.01	5.6±0.27	18.1±1.35	10.62±1.25	3178±362	0.3±0.02
175	42.1±0.71	21.9±1.44	67±0.9	8.7±0.45	14±0.86	10.32±1.98	2294±381.5	0.2±0.01
203	39.4±0.49	22.8±0.2	64.8±0.55	8.9±0.16	15.5±0.48	10.74±0.48	2427±53.6	0.2±0.01

6.5 DISCUSSION

6.5.1 Microbial succession

In agreement with Chapter 4 and several other studies (e.g. Hermann and Shann 1997; Klamer and Bååth 1998; Steger et al. 2003), the Gram-positive bacteria were most prominent during the heat peaks, while the proportion of fungi clearly decreased with increasing temperature and the Gram-negative bacteria peaked during the intermediate temperature drop (day 7) in the thermophilic phase. Increased proportions of Actinomycetes in the later compost stages were also found by Hellmann et al. (1997), Klamer and Bååth (1998), Bolta et al. (2003) and Steger et al. (2003), and might be explained by their slower development compared to most bacteria and fungi and their ineffectiveness as competitors when nutrient levels are high at the beginning of the process (Beffa et al. 1996). However, unlike in chapter 4 and several other studies (e.g. Herrmann and Shann 1997; Klamer and Bååth 1998; Steger et al. 2003; Ryckeboer et al., 2003), but similar to Bolta et al. (2003) and recurrent in all three heaps studied here, the proportions of the fungal PLFA did not increase but rather decreased at the end of the process, even when near ambient temperature levels were reached. It is highly unlikely that this could be completely attributed to the possible effects of previous elevated temperatures on fungal growth or suppressive (top-down) control of the abundant fungal-feeding nematodes on the fungi. Alternatively, the variable outcome of our PLFA study could be partly ascribed to the technique to characterize the fungal community. The amount of reliable fungal biomarkers is rather limited: only two reliable markers were detected and analyzed by the GCMS (i.e. 18:1ω9c and 18:2ω6,9) compared to 15 bacterial biomarkers (Joergensen and Wichern 2008). Although the applied technique does not provide any information on this matter, diversity might also be an important factor here. The fungal diversity is known to increase with duration of composting (Ryckeboer et al. 2003) and it is not impossible that some forms or species of fungi appearing more at the end of the process are not detected with current PLFA techniques.

6.5.2 Nematode succession

Clear shifts in the nematode community structure were found in the farm compost processes. These shifts are not clearly delineated according to the three typical successive temperature related composting phases, i.e. thermophilic, cooling and maturation phase, as previously described in Chapters 2 and 4. Hence, although the nematode patterns in the three processes are remarkably similar, the nematode succession is less straightforward compared to previous studies. But most importantly, the absence of a linear relation of temperature and time in

current study facilitated to distinguish between the effect of temperature and duration of composting. Although the timing of the nematode succession is different compared to previous studies, the general patterns are largely similar. The nematode successions are largely represented by the same functional groups and nematode taxa, i.e. during and directly after the heat peaks (day 0-10) bacterial feeding enrichment opportunists are most important, including *Pelodera* sp. together with *Rhabditella axei*. Subsequently, the bacterial-feeding/predator community (days 17-49) became dominant. Then, from day 63-133, the proportion of the fungal-feeding general opportunists initially increased. However, with increasing temperatures this ratio decreased again resulting in a less unambiguous positive linear relation with compost time compared to former studies (Chapters 2 and 4).

Since an increasing share of fungal-feeding nematodes was suggested as a potential tool to assess compost maturity (Chapters 2 and 4), the relation of fungal-feeding nematodes with time, temperature and microbial community was better elaborated here. The density of fungal-feeding nematodes is, like the total density of nematodes, significantly related to temperature, but also to duration of composting. A model including these parameters predicts the observed fungal-feeding nematodes fairly well (Figure 6.8A), except for the moments after the thermophilic phase (i.e. days 17-35). These are the samples where the bacterial-feeding/predators (i.e. *Mononchoides composticola*) were dominant and possibly outcompeted fungal-feeding nematodes due to their ability to feed on bacteria, other compost nematodes (Chapter 3) and most likely also fungi (note that a fungal-feeding behavior has also been reported for *Pristionchus pacificus* of the same nematode family (Fürst von Lieven and Sudhaus 2000)). Nematode densitieswere highly dependent on temperature. In contrast, the F/F+B ratio was only significantly positively related with duration of composting. Hence, our results show that this ratio, previously proposed as an indicator of compost maturity, is effectively related to the duration of a compost process and is less affected by temperature fluctuations. Nevertheless, the model that included duration of composting and temperature predicted higher F/F+B values than observed at sampling moments with relatively high temperatures resulting in low nematodes densities (Figure 6.8B). This is due to the fact that the ratio, being a proportion, is not sensitive to densities per se. Compared to the changes in the nematode data, the PLFA data are more or less stable, especially towards the second half of the process. Thus, when the amount of fungal PLFA or the PLFA based F/B ratio were added as explanatory variables in the models of the abundances of fungal-feeding nematodes and of the F/F+B ratio, no additional information was explained. Surprisingly, during maturation, the increased levels of fungal-feeding nematodes did not coincide with increased levels of fungal PLFA. Similarly,

Sánchez-Moreno et al. (2006) reported that in soil the fungal-feeding nematodes were not related as strongly with ergosterol as expected. Despite the different proxies used for fungal biomass, i.e. ergosterol *vs.* PLFA, these two findings suggest that fungal biomass may not be determining the size of the fungal feeder populations (Sánchez-Moreno et al. 2006).

6.5.3 Green waste compost versus farm compost

The farm composts were well balanced replicated processes that allowed a founded modeling of nematode parameters, whereas the green waste compost represents a standardized industrial process. Both abiotic (i.e. temperature) and biotic (i.e. nematode and microbial community succession) data were manifestly different. The persistent high temperatures during the industrial green waste composting can be partly related to the feedstock material. The green waste compost was composed of less ligneous materials (i.e. grass hay, straw of clover) and more fresh materials (i.e. fresh grass, mixed green waste), and the latter contain more readily degradable carbon which causes prolonged periods of very high temperatures as a result of decomposition by mainly thermophilic bacteria (Steger et al. 2005). The nematode abundances and diversity were significantly lower and the nematode community of the “finished” green waste compost hardly contained fungal-feeding nematodes. Both the amount of fungal-feeding nematodes and the F/F+B ratio are clearly lower than predicted by the models based on more balanced composts (Figure 6.7), respectively 1 fungal-feeding nematode *vs.* 41 predicted in 100g DW compost and a ratio of 0.003 *vs.* 0.1 predicted. The very low F/F+B ratio is an indication that the composting process has not yet been able to mature towards a balanced biological community. This is quite evident given the persistently high temperatures. We endorse that in such extreme situations nematode based in indices appear somewhat superficially, they could be of more relevance for more subtle different situations. Nevertheless, especially compared to the farm composts, the green waste compost is extremely poor in nematode biology. In addition, since both the abundance and (functional) diversity of the nematode community together with the availability of an adequate resource are important for the continuity of their ecosystem services in soil (Ferris 2010), the structure and quality of the nematode community in the finished composts may be an important issue for the beneficial effects of compost when added to the soil.

6.5.4 Conclusion

In conclusion several features of the nematode community during composting (i.e. nematode abundances, total amount of fungal-feeding nematodes and the F/F+B ratio) showed a clear and consistent pattern in all three independent farm processes. This made it possible to design models for each of these features. Especially the nematode based F/F+B holds promise as a reliable indicator of compost maturity since it was not affected by temperature fluctuations but only related with duration of composting. The next step is to improve the obtained models based on other compost methods and validate their universal use as an effective indictor of compost maturity.

Acknowledgements

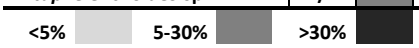
Hanne Steel acknowledges the Research Foundation - Flanders (FWO-Flanders, Belgium) for providing a PhD grant as aspirant. David Buchan is the recipient of a PhD grant from the Special Research Fund of Ghent University (BOF project 01J16907). We are grateful to Koen Willekens and Bart Vandecasteele for providing the opportunity to sample the composting processes at ILVO.

6.6 SUPPLEMENTARY MATERIAL

Supplementary Table 6.S1

Nematode species list with indication of relative abundance of the species during the Farm 1 composting process (averages of 3 replicates). The feeding type (FT) of each species is also provided (3= bacterial-feeding, 2= fungal-feeding, 3-5a= bacterial-feeding/predator, 5= predator).

Taxa	FT	Day of compost duration															
		0	3	7	10	17	24	35	49	63	77	105	119	133	147	175	203
Cylindrocorpus sp.	3																
Pelodera teres	3																
Rhabditella axei	3																
Rhabditis sp.	3																
Pelodera sp.	3																
Choriorhabditis sp.	3																
juv. Rhabditidae	3																
Acrostichus sp.	3																
Halicephalobus cfr. *gingivalis*	3																
juv. Diplogasteridae	3																
Panagrolaimus sp.	3																
Pelodera cylindrica	3																
Protorhabditis sp.	3																
Mesorhabditis sp.	3																
Diplogasteritus sp.	3																
Fam. Bunonematidae	3																
Cephaloboides sp.	3																
Fam. Panagrolaimidae	3																
Diploscapter coronatus	3																
Poikilolaimus sp.	3																
Eucephalobus sp.	3																
juv. Rhabditidae /P	3																
Teratorhabditis sp.	3																
Teratocephalus sp.	3																
Fam. Tylenchidae	2																
Aphelenchoides sp.	2																
Ditylenchus sp.	2																
Fam. Neotylenchidae	2																
Butlerius sp.	3-5a																
Fictor sp.	3-5a																
Mononchoides composticola	3-5a																
juv. Diplogasteridae /M	3-5a																
Seinura sp.	5																
Ektaphelenchoides sp.	/																



Supplementary Table 6.S2

Nematode species list with indication of relative abundance of the species during the Farm 2 composting process (averages of 3 replicates). The feeding type (FT) of each species is also provided (3= bacterial-feeding, 2= fungal-feeding, 3-5a= bacterial-feeding/predator, 5= predator, 8= omnivorous).

Taxa	FT	Day of compost duration															
		0	3	7	10	17	24	35	49	63	77	105	119	133	147	175	203
Pelodera sp.	3																
Protorhabditis sp.	3																
Rhabditella axei	3																
Diplogasteritus sp.	3																
Teratorhabditis sp.	3																
juv. Rhabditidae	3																
Choriorhabditis sp.	3																
juv. Diplogasteridae	3																
Acrostichus sp.	3																
Mesorhabditis sp.	3																
Fam. Panagrolaimidae	3																
Pelodera cylindrica	3																
Halicephalobus cfr. *gingivalis*	3																
Poikilolaimus sp.	3																
Teratocephalus sp.	3																
Cephaloboides sp.	3																
Panagrolaimus sp.	3																
juv. Diplogastridae/M	3																
Fam Bunonematidae	3																
Crustorhabditis sp.	3																
Diploscapter coronatus	3																
Eucephalobus sp.	3																
juv. Rhabditidae/P	3																
Ditylenchus sp.	2																
Aphelenchoides sp.	2																
Fam. Neotylenchidae	2																
Fam. Tylenchidae	2																
Diplogaster sp.	3-5a																
Fictor sp.	3-5a																
Mononchoides composticola	3-5a																
Butlerius sp.	3-5a																
Seinura sp.	5																
Fam. Qudsianematidae	8																
Ektaphelenchoides sp.	/																

<5% 5-30% >30%

Supplementary Table 6.S3

Nematode species list with indication of relative abundance of the species during the Farm 3 composting process (averages of 3 replicates). The feeding type (FT) of each species is also provided (3= bacterial-feeding, 2= fungal-feeding, 3-5a= bacterial-feeding/predator, 5= predator, 8= omnivorous).

Taxa	FT	Day of compost duration															
		0	3	7	10	17	24	35	49	63	77	105	119	133	147	175	203
Crusthorhabditis sp.	3																
Pelodera teres	3																
Choriorhabditis sp.	3																
Poikilolaimus sp.	3																
juv. Rhabditidae	3																
juv. Diplogasteridae	3																
Rhabditis sp.	3																
Rhabditella axei	3																
*Acrostichus*sp.	3																
Protorhabditis sp.	3																
Diplogasteritus sp.	3																
Mesorhabditis sp.	3																
Myolaimus sp.	3																
Pelodera cylindrica	3																
Pelodera sp.	3																
Plectus sp.	3																
Halicephalobus cfr. *gingivalis*	3																
Operculorhabditis sp.	3																
Cylindrocorpus sp.	3																
Diploscapter coronatus	3																
Eucephalobus sp.	3																
Ditylenchus sp.	2																
Fam. Tylenchidae	2																
Aphelenchoides sp.	2																
Fam. Neotylenchidae	2																
Diplogaster sp.	3-5a																
Butlerius sp.	3-5a																
Mononchoides composticola	3-5a																
juv. Diplogasteridae /M	3-5a																
Seinura sp.	5b																
Fam. Qudsianematidae	8																

<5% 5-30% >30%

Supplementary Table 6.S4

Nematode species list with indication of relative abundance of the species during the green waste composting process (averages of 3 replicates). The feeding type (FT) of each species is also provided (3= bacterial-feeding, 2= fungal-feeding, 3-5a= bacterial-feeding/predator, 5= predator, 8= omnivorous).

TAXA	FT	Day of compost duration				
		0	33	39	61	83
Acrostichus sp.	3					
Teratorhabditis sp.	3					
Panagrolaimus sp.	3					
Rhabditella axei	3					
Oscheius sp.	3					
juv. Rhabditidae	3					
Pelodera sp.	3					
juv. Panagrolaimidae	3					
juv. Diplogasteridae	3					
Cephalobus sp.	3					
Diploscapter coronatus	3					
Halicephalobus cfr. gingivalis	3					
Fam. Panagrolaimidae	3					
Aphelenchoides sp.	2					
Mononchoides sp.	3-5a					
Seinura sp.	5b					
Fam. Dorylaimidae	8					

<5% 5-30% >30%

6.7 REFERENCES

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresource Technol. 74, 35-47.

Been, T.H., Schomaker, C.H., 2006. Distribution Patterns and Sampling. In: Perry, R.N., Moens, M. (eds.), Plant Nematology, CABI publishing, Wallingford, pp. 302-326.

Beffa, T., Blanc, M., Marilley, L., Lott Fischer, J., Lyon, P.-F., Aragno, M., 1996. Taxonomic and metabolic microbial diversity during composting. In: de Bertoldi, M., Sequi, P., Lemmes, B., Papi, T. (Eds.), The Science of Composting . Blackies Academic and Proffessional, Glasgow, Scotland, pp. 149-161.

Bolta, S.V., Mihelic, R., Lobnik, F., Lestan, D., 2003. Microbial community structure during composting with and without mass inocula. Compost Sci. Util. 11, 6-15.

Clarke, K.R., Warwick, R.M., 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E: Plymouth.

Cogger, C.G., 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Sci. Util. 13, 243-251.

Cooperland, L.R., 2000. Composting: art and science of organic waste conservation to a valuable soil resource. Lab. Med. 31, 283-290.

Hellmann, B., Zelles, L., Palojarvi, A., Bai, Q.Y., 1997. Emission of climate-relevant trace gases and succession of microbial communities during open-window composting. Appl. Environ. Microb. 63, 1011-1018.

Herrmann, R.F., Shann, J.F., 1997. Microbial community changes during the composting of municipal solid waste. Microb. Ecol. 33, 78-85.

Klamer, M., Bååth, E., 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. Fems Microbiology Ecology 27, 9-20.

Kuo, S., Ortiz-Escobar, M.E., Hue, N.V., Hummel, R.L., 2004. Composting and Compost Utilization for agronomic and container crops. Recent Res. Devel. Environ. Biol. 1, 451-513.

Neher, D.A., 2001. Role of Nematodes in Soil Health and their Use as Indicators. Journal of Nematology 33, 161-168.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Ann. Microbiol. 53, 349-410.

Sanchez-Moreno, S., Minoshima, H., Ferris, H., Jackson, L.E., 2006. Linking soil properties and nematode community composition: effects of soil management on soil food webs. Nematology 8, 703-715.

Steger, K., Jarvis, A., Smars, S., Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. J. Microbiol. Meth. 55, 371-382.

Steger, K., Eklind, Y., Olsson, J., Sundh, I., 2005. Microbial community growth and utilization of carbon constituents during thermophilic composting at different oxygen levels. Microb. Ecol. 50, 163-171

Young, C.-C., Rekha, P.D., Arun, A.B., 2005. What happens during composting. In: Chen, Z.S. (Ed), Compost production: a manual for Asian farmers, Food & Fertilizer Technology Center, Taipei, Taiwan, pp. 8-19.

Zvomuya, F., Larney, F.J., DeMaere, P.R., Olson, A.F., 2008. Organic amendment effects on crop productivity and nutrient uptake on reclaimed natural gas wellsites. Nutr. Cycl. in Agroecosys. 80, 223-232.

7. Nematode communities and macronutrients in composts and compost-amended soils as affected by feedstock composition

Modified from:

Steel H, Vandecasteele B, Willekens K, Sabbe K, Moens T and Bert W (2012). Nematode communities in composts and compost-amended soils as affected by feedstock composition. Applied Soil Ecology 61: 100-112.

7.1. ABSTRACT

Farm composts were produced from selected feedstocks and compared to one external farm compost and one green waste compost. Nematodes were omnipresent in the composts (on average 26 ind./g dry weight compost) and many taxa were common to all composts (i.e. *Mononchoides composticola*, *Diploscapter coronatus*, *Halicephalobus gingivalis*, *Ditylenchus* sp., *Diplogaster* sp., *Diplogastrellus* sp. and *Diplogasteritus* sp.) and occurred in similar proportions. Composts with a somewhat different species composition or proportion also displayed differences in the abiotic parameters, suggesting that compost maturity and status may be reflected by the nematode community. Significant differences (ANOSIM, $p = 0.001$) in nematode community between the farm composts and the green waste compost were found. Nevertheless, despite largely different feedstock materials and proportions, and despite clear differences in chemical properties and stability of the composts, biologically very similar composts were produced. The 11 composts of different composition were further tested in an incubation experiment to investigate their short-term effect on the existent soil nematode community and on the soil chemical properties. Therefore, the nematode community together with several abiotic parameters (pH, EC, DM, OM, Ptot, C/N, and NO_3/NH_4) of the used composts and the soils before and 12 weeks after compost amendment, were analyzed. All compost amendments resulted in a significant pH increase in the soil (one-way ANOVA, $p < 0.01$), and differences in plant-available macronutrients in the soil between the applied composts were detected. The changes in soil chemical properties after compost addition were related to the compost type and could be linked to the properties of the used feedstock materials. After 12 weeks of incubation, the nematode numbers in the soil increased, except in soil amended with the green waste compost. In particular, the abundance of the fungal-feeding *Ditylenchus* increased significantly (one-way ANOVA, $p < 0.03$) in all soils amended with farm compost. This increase may well be purely caused by the addition of nematodes present in the compost.

7.2. INTRODUCTION

Composting is an effective tool for the management of municipal and agro-industrial wastes by converting raw organic materials into a stabilized form, destroying human and animal pathogens and recycling valuable plant nutrients (Naidu et al. 2004). Multiple benefits of compost addition to the soil's physical and chemical characteristics have been reported and include increased soil organic matter content (Termorshuizen et al. 2004) and the subsequent improvement of soil

aeration, soil porosity, drainage, and water holding capacity (Cogger 2005; Kuo et al. 2004; Termorshuizen et al. 2004). Furthermore, compost can provide an important source of nutrients for plants by providing readily available nitrogen and by promoting mineralization of compost nitrogen in the soil (Cogger 2005; D'Hose et al. 2012; Zvomuya et al. 2008). Composts are also known for their disease-suppressive activity based on both physicochemical and biological mechanisms (Akhtar and Malik 2000; Bailey and Lazarovit, 2003; Kuo et al, 2004; Oka 2010; Reuveni et al. 2002). Another benefit of compost application is the suppression of weed seeds (De Cauwer et al. 2010, 2011). Therefore, truly functional compost should meet criteria for stability and maturity before application (Zorpas 2009).

The use of compost significantly impacts the soil fauna and flora (Pfotzer and Schüler 1997). Application of compost enhanced the biological activity of the soil (i.e. higher microbial activity and higher numbers of protozoa and bacterial-feeding nematodes), resulting in enhanced turnover of organic matter and release of plant available nutrients (Forge et al. 2003). In translocating and transforming litter and soil organic matter and in changing the supply of nutrients to plant roots, soil fauna fulfill crucial roles in the nutrient supply and the conservation of the soil structure (Brussaard et al. 2004). Hence, compost application can strengthen the role of the living soil. However, current insight into the consequences of compost addition for the complex interactions in the soil is limited. In addition, soil biota can be considered important soil quality indicators. The nematode community in particular is strongly influenced by soil physical and chemical properties (Boag and Yeates 1998; Goralczyk 1998). Furthermore, there is considerable evidence that organic amendments cause changes to the whole soil nematode community, affecting the presence and abundance of different nematode groups (Bulluck et al. 2002; Nahar et al. 2006; Renčo et al. 2010). Recently a pilot study highlighted the potential of the nematode community as an indicator of compost status (Chapter 2). The soil nematode community, in turn, plays an important role in nutrient cycling because of its abundance, rapid life cycle and intricate interactions with soil microbes and predators (Ingham et al. 1985).

To date, only few studies have investigated the effect of compost on the soil food web (Bulluck et al. 2002; Leroy et al. 2007; Nahar et al. 2006; Renčo et al. 2010). To the best of our knowledge, in all these studies compost was treated as a black box, i.e. without or with little a priori knowledge on compost biotic properties, especially regarding the nematode community. Nevertheless, composts may vary significantly in chemical, physical and biological properties due to differences in feedstock composition, process and conditions of compost storage. The variation in compost composition, stability and maturity determines its effect on soil properties.

It is important to know to what extent effects of composts on soil biology are related to the communities living in the compost at the time of its application to soil.

In this study nematode abundance and community composition were analyzed together with chemical properties of different types of compost. Nine composts were produced on-farm from selected feedstock materials to obtain compost types with variable organic matter contents and concentrations of nutrients. These 9 composts, and 2 other composts obtained from other facilities, were thoroughly characterized chemically (especially concentrations of the macronutrients Ca, K, Mg and Na) and biologically (i.e. the nematofauna and fungal biomass). The overall objective of this study is to integrate a comprehensive picture of the factors determining nematode assemblage in compost and of how application of this compost with its associated nematodes affects soil nematode communities and their abiotic environment. To our knowledge, this study is the first to take into account the whole process, from the preparation of a compost to its effects on the soil biota. This main objective translates into the following specific hypothesis: (1) the selection of feedstock materials (e.g. wood chips vs. bark) would influence the nematode community and/or the chemical properties of composts; (2) the short-term (12 weeks long) effect of compost application on the macronutrients concentrations and the nematodes in the soil would be different for the different types of compost; and (3) the effects of compost application on the existent soil nematode community should be related to chemical compost properties.

7.3. MATERIALS AND METHODS

The effect of feedstock composition on nematode community composition, nematode abundance and on compost chemical properties was investigated in a two-stage experiment consisting of composting trials (Section 7.2.1) and a soil incubation experiment (Section 7.2.2) using the composts obtained in the composting trials. The methods listed in Sections 7.2.3 and 7.2.4 were applied in both stages of the experiment.

7.3.1. Compost trials

Three separate compost trials were executed in open air on a concrete floor at the experimental farm of the Institute for Agricultural and Fisheries Research (ILVO at Merelbeke, Belgium) (Figure 7.1). Each trial consisted of three different feedstock compositions. Selection of the feedstock materials for the compost trial treatments was based on the availability of on-farm waste materials (e.g. manure, hay, crop residues) and on the approximation of the proportion of 60%

brown and 40% green material as ideal starting point (http://compost.css.cornell.edu/OnFarmHandbook/onfarm TOC.html).

For the first trial, three compost windrows were set up (3-5 January 2007) with 31.5% on a volume to volume base (%, v/v) grass hay and 63.5% (v/v) ground tree bark. Three types of tree bark were compared in these piles: oak (*Quercus robur*), poplar (*Populus* sp.) and Norway spruce (*Picea abies*), respectively. Additionally, 5% of poultry manure, mixed with wood shavings, was added. After 17 weeks of composting, the composts were further stored in piles for another 35 weeks.

For the second trial, three compost piles were set up (11-13 February 2008) with different ratios of grass hay and straw of clover on one hand, and ground poplar bark on the other, i.e. 25/75% (v/v), 50/50% (v/v) and 75/25% (v/v), respectively. The amount of grass hay in the three compost piles was 0, 23 and 46% (v/v), respectively. Additionally, urea was added after 2 weeks to correct the C/N content of the feedstock towards 30/1, which is generally considered an ideal starting ratio for composting (Zorpas 2009). After three weeks, cane molasses and spoiled ensilaged maize were mixed in the feedstock as a compost starter. After 16 weeks of composting, the composts were further stored in piles for another 16 weeks.

For the third trial, three compost piles were set up (2-4 March 2009) with 7% (v/v) crop residues of leek, 27% (v/v) of straw of grass, 16% (v/v) of spoiled ensilaged maize (as compost starter) and 50% (v/v) of ground tree bark or wood chips. Wood chips of willow, wood chips of poplar and ground poplar bark were used in the first, second and third windrow, respectively. The wood chips were harvested at the short rotation plantation at ILVO. No storage period was included in this trial.

In all trials, the feedstock materials were mechanically mixed with a windrow turner and composted in a windrow system with a length of 10 m per compost type. Temperature and CO_2 levels in the composts were monitored and when necessary, the composts were mixed with a Tractor-pulled Sandberger ST300 windrow compost turner and covered (with Top tex compost fleece) or rewetted.

After 52, 32 and 24 weeks respectively (cf. Figure 7.1), three bulk samples were taken from each trial treatment. Each bulk sample consisted of 10 individual samples (±3 l) which were lumped together. These were then sieved over a 20 mm mesh and the fraction <20 mm was stored in the refrigerator at 4 °C.

In addition to the 3 × 3 trial treatments, we also examined nematode composition of two additional composts: i.e. commercially available green waste compost and farm compost from another research station (i.e. external farm compost).

7.3.2. Incubation experiments

For each compost trial treatment with finished composts, an incubation experiment was set up (Figure 7.1) in which agricultural top soils were mixed with the different composts. Incubation experiments were performed in 5 l vessels with 4 replicates per treatment. Top layer material from soils under agricultural practice was used. For these initial soils both the chemical properties (Table 7.1) and the nematode community (except for trial 1) (Table 7.2) were characterized. Per vessel the equivalent of 50 tons fresh compost per ha was applied on a surface basis: 0.117 kg fresh compost was mixed with

1.64 kg dry mass of soil after adjusting the soil moisture content to 50% water-filled pore space. Soil bulk density after filling the vessels was 1.4 kg dm^{-3}. The vessels were closed with a cover provided with a small split for exchange of air. The vessels were kept for 12 weeks at 17 ±2 °C, and the soil water content was adjusted every two weeks.

In addition, the green waste and farm compost (see Section 7.2.1) were included in the incubation experiments performed in 2008 (expt. 2) and 2009 (expt. 3) respectively.

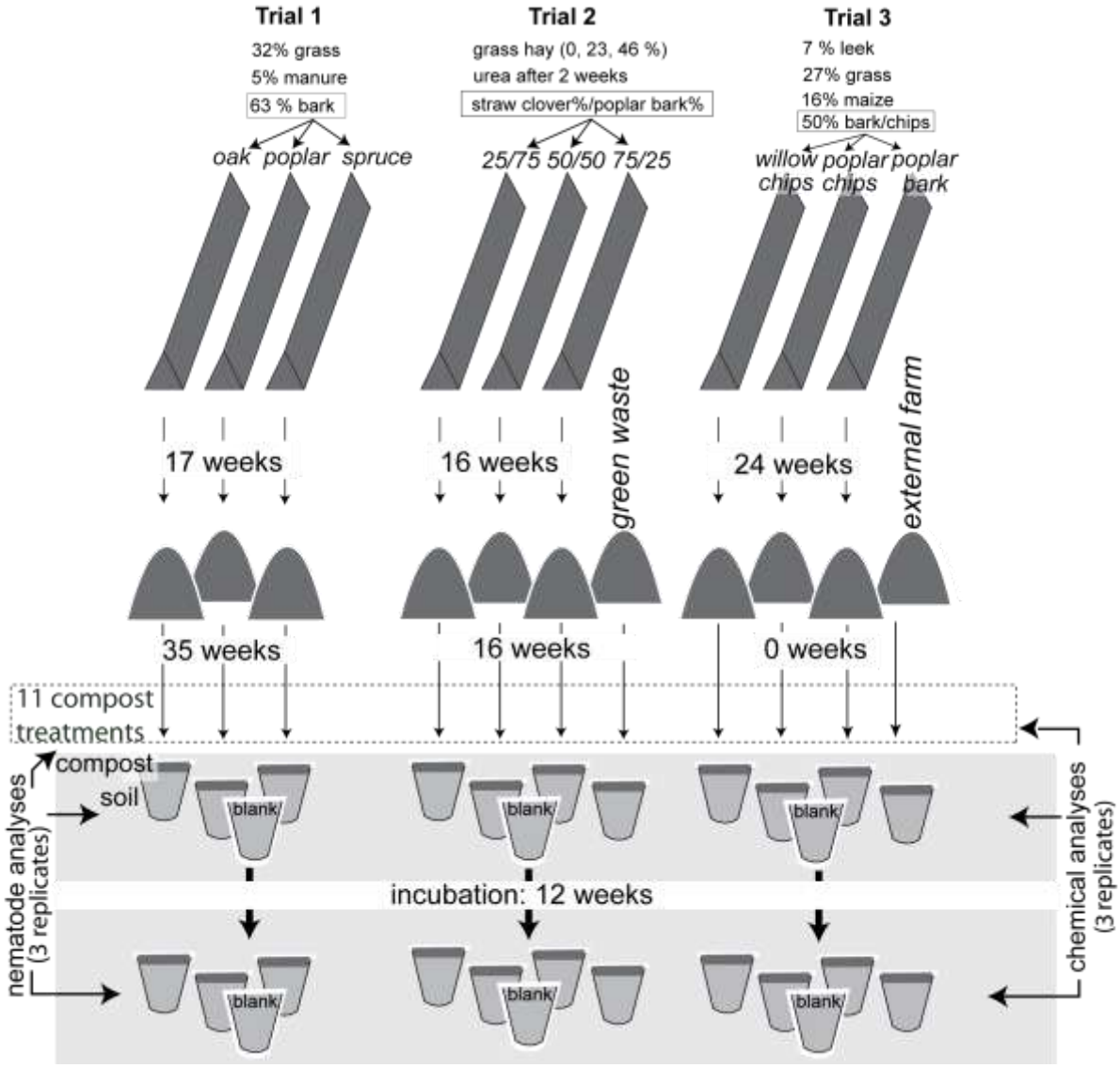


Figure 7.1:

Experimental setup of the two-stage experiment. The upper level indicates which feedstock materials were used in which proportion and gives information about the time interval between the phases of the three composting trials. The lower level (in gray) displays the set up of the three incubation experiments. Arrows indicate which analyses were done on which samples.

Table 7.1:

Chemical properties of the soils used in the incubation trials (PAL: ammonium lactate extractable P, OC: organic carbon). Values illustrated are means (± SD) of 4 replicates.

	PAL (mg/100g dry soil)	pH-KCl	NO_3-N (mg/kg dry soil)	NH_4-N (mg/kg dry soil)	OC (%/dry soil)
Incubation 1	16.2 (0.9)	5.28 (0.07)	15.5 (0.9)	1.9 (0.3)	1.1 (0.1)
Incubation 2	51.3 (8.7)	6.44 (0.02)	11.5 (0.6)	1.8 (0.1)	1.6 (0.02)
Incubation 3	8.8 (0.9)	5.00 (0.04)	60.6 (6.3)	3.5 (0.3)	0.9 (0.02)

Table 7.2:
Nematode community of soils used in incubations with compost of trials 2 and 3 (Incubation 2 and Incubation 3 respectively). Average percentage of 3 samples per taxa with standard deviation in parentheses.

%	Incubation 2	Incubation 3
Fam. Rhabditidae	8.3 (0.8)	11.8 (3.6)
Fam. Cephalobidae	28.2 (3.8)	13.6 (2.7)
Fam. Aphelenchidae	0	18.4 (2.9)
Fam. Tylenchidae	4 (0.4)	22.9 (6.8)
Fam. Neotylenchidae	0	4.3 (1.7)
Ordo Dorylaimida	26.7 (2)	0.1 (0.1)
Tylenchorhynchus sp.	6.5 (3.4)	0
Pratylenchus sp.	15 (1.4)	0
Ditylenchus sp.	4.6 (1.7)	27.5 (4)
Rotylenchus sp.	4.7 (2)	0
Mononchoides sp.	0.9 (0.9)	0.6 (1)
Steinernema sp.	1.2 (0.58)	0
Diplogasteritus sp.	0	0.4 (0.6)

7.3.3. Nematode community analyses

The nematode communities of the compost (Section 7.2.1) and incubation (Section 7.2.2) experiments were analyzed. From each of the 33 (3 ×11) compost bulk samples (Section 7.2.1), 300 ml subsamples were taken and analyzed. For the incubation experiments, 200 ml of each of the three replicates of the initial soils (incubations 2 and 3 with compost of trials 2 and 3 respectively, see Figure 7.1), the soils after the incubation experiments and the untreated soils (Section 7.2.2), were analyzed. We used a modified Baermann funnel method (tray 49 cm × 37.5 cm, basket 38.5 cm × 19.5 cm) (Hooper 1986) for nematode extraction. Total nematode numbers were counted using a stereomicroscope (Leica MZ95). For light microscopy observations the specimens were collected in a very small drop of water in a staining block. Formaldehyde (4% with 1% glycerol) was heated to 70 °C and an excess (4-5 ml) was quickly added to the specimens to kill and preserve the nematodes (Seinhorst 1966). The preserved nematodes were processed to anhydrous glycerol following the glycerol-ethanol method (Seinhorst 1959, as modified by De Grisse 1969) and mounted on mass glass slides (slide 40 mm × 76 mm; cover glass 34 mm × 60 mm). Measurements and identification were done manually with a camera lucida on an Olympus BX 51 DIC microscope (Olympus Optical, Tokyo, Japan). For each subsample at least 100 nematodes were identified to family and genus whenever possible. The abundance (individuals/gram dry weight compost or soil) of each taxon in each sample was calculated based on the density and moisture content of the compost or soil. Nematode genera were assigned to the "coloniser-persister" cp-scale according to their *r* vs. *K* life-strategy characteristics (Bongers 1990, 1999) and were also classified according to their feeding type

(Yeates et al. 1993). These allocations were, respectively, used to calculate the sum Maturity Index (MI) (Bongers 1990, 1999), the f/b (fungal to bacterial-feeder) ratio and the Channel Index (CI). The f/b ratio and CI were used as indicators of the dominant decomposition pathway (Ferris et al. 2001; Ruess 2003).

7.3.4. Chemical analyses of compost, blank soil and incubated soil

Chemical analyses were performed for the compost trial treatments (3 subsamples for each of the 11 bulk samples, corresponding with the samples taken for the nematode analyses, cf. Section 7.2.3) and all replicates of the incubation experiments.

Sample preparation of composts for chemical analyses, determination of dry matter content (DM), moisture content and laboratory compacted bulk density were performed according to EN[8] 13040. Electrical conductivity (EC) (EN 13038) and pHH2O (EN 13037) in the compost samples were measured in a 1:5 soil to water suspension. Determination of organic matter (OM) and ash content in the composts was done according to EN 13039. Extraction of water-soluble nutrients and elements was performed according to EN 13652, and bulk elemental concentrations were measured with a Dionex DX-600 IC ion chromatograph (Dionex, Sunnyvale, CA). Ca, K, Mg and P were extracted in ammonium acetate and measured by simultaneous Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, VISTA-PRO, Varian, Palo Alto, CA). Compost samples were dried for 4 days at 70 °C, mechanically ground in a cross beater mill (SK100, Retsch, Haan, Germany) equipped with heavy-metal-free grinding tools. Determination of organic matter (OM) and ash content in the composts was done according to EN 13039. Total N concentration in the compost samples was determined according to the modified Kjeldahl method (EN 13654-1). Pseudo-total element concentrations are measured by simultaneous ICP-OES, following ashing and digestion with HNO_3 (p.a. 65%).

As an indicator of compost stability, oxygen uptake rate (OUR) at 20 °C was calculated from the oxygen consumption due to microbial activity of 20 g compost (<1 cm fraction) in 200 ml buffered nutrient solution during 5 days of shaking in a closed Oxitop respirometer. OUR is expressed as mmol/kg OM/h. Ergosterol as a proxy for fungal biomass in the freeze-dried compost samples was measured with a Thermo Finnigan Surveyor HPLC with UV-VIS detector and a Waters Nova-Pak C18 4-µm column, after extraction with methanol based on physical disruption (Gong et al. 2001).

[8] EN: European Standard. European Standards are developed by CEN, the European Commission for Standardization. Numbers refer to the specific protocols.

After twelve weeks the soils in the incubation vessels were sampled destructively and mineral nitrogen content (both NO_3-N and NH_4-N) was extracted in a 1 M KCl solution according to ISO[9] 14256-2 and measured with a Foss Fiastar 5000 continuous flow analyzer. Soil moisture content (expressed on a fresh weight basis) was determined as the weight loss at 105 °C after (at least) 24 h. pH was measured in a 1 M KCl solution according to ISO 10390. Ammonium lactate extractable P (PAL), K, Mg, Ca and Na were determined by shaking 5 g air-dried soil in 100 ml ammonium lactate for 4 h (Egnèr et al. 1960), and measured with a simultaneous ICP-OES (VISTA-PRO, Varian, Palo Alto, CA). The blank soil at the start of the incubation experiment was analyzed similarly, and the organic carbon content was measured with a Skalar Primacs SLC TOC-analyzer according to ISO 10694.

7.3.5. Statistical analyses

Compost trials

Differences in abiotic parameters, nematode densities and nematode-based indices (ΣMI, CI and f/b ratio) between different composts were analyzed using one-way ANOVA in Statistica 6.0 (Statsoft Inc.); differences in their nematode community composition were analyzed using Analysis of Similarities (ANOSIM) and Similarity Percentages analysis (SIMPER) in Primer 6 (Clarke and Warwick 2001). The relationship between variation in nematode community composition and the measured abiotic parameters was analyzed using ordination techniques in Canoco for Windows, version 4.5 (ter Braak and Smilauer 1998).

ANOVA assumptions were tested using the Kolomogorov-Smirnov statistic for normality and Levene's test for homogeneity of variances. To fit the assumptions the nematode densities and the CI data were square-root transformed while the f/b ratio data were log-transformed. All one-way ANOVA analyses were followed by post hoc pair wise comparisons using Tukey's HSD. However, it should be noted that because of the scale of the compost heaps and the duration of the composting process in the on-farm composting trials (Section 7.2.1), proper replication of compost types and trials was not feasible. Hence, replicate samples were taken from single compost heaps and are thus essentially pseudoreplicates. Furthermore, although the duration of composting in the different trials was not identical, we analyzed data of the three different trials in one analysis to be able to compare all composts, which was one of the prime goals of this study.

The ANOSIM analyses were performed comparing green vs. farm composts, trials and used feedstock materials, and were based on Bray-Curtis similarity matrices of square-root

[9] ISO: International Organization for Standardization. Numbers refer to specific approved protocols by ISO.

transformed abundance data. SIMPER was used to identify which species were primarily responsible for the differences between nematode assemblages.

For the ordination analysis a preliminary Detrended Correspondence Analysis (DCA) using detrending by segments revealed a weak turnover in species composition between the samples (length of gradient < 2.5 SD, cf. Jongman et al. 1995). Hence, we used a linear constrained technique, viz. Redundancy Analysis (RDA), for our analyses. The data set consisted of the log(x + 1) transformed abundances (numbers of individuals/gDM) of 11 nematode taxa in 28 samples. Only one replicate sample of the external farm compost was retained and the green waste compost was excluded due to missing values. The forward selection procedure in RDA was used to select only those variables that contributed significantly to the variation in the nematode data using Monte Carlo permutation tests (4999 permutations). The remaining, non-selected abiotic variables were introduced as supplementary (passive) variables.

Spearman rank correlations between mean relative bacterial-feeding nematode abundances, fungal-feeding nematode abundances and ergosterol concentrations in the composts were calculated in Statistica 6.0.

Incubation experiments

Differences in the effect of compost addition on abiotic soil properties (mineral nitrogen content (Nmin), pH-KCl and ammonium lactate extractable P, K, Mg, Ca and Na), nematode densities and nematode based indices (ΣMI, CI and f/b ratio) were analyzed using one-way ANOVA in Statistica 6.0 (Statsoft Inc.); the effects on the soil nematode community were analyzed using MANOVA in PERMANOVA (PRIMER).

Separate analyses were performed for each incubation experiment. One-way ANOVA analyses and subsequent post hoc Tukey HSD tests were performed on log-transformed data for K, Na, P, Nmin and total nematode numbers and on square-root transformed data for ΣMI, CI and f/b ratio. Due to the small sample size (i.e. 3 replicates per treatment for the nematode data), ANOSIM was not able to locate the significant differences in nematode communities of the overall test using pair-wise tests. Therefore, a one-way permutational MANOVA (PERMANOVA) with compost as a fixed factor was performed. Monte Carlo p-values were used to indicate significant differences in the pair-wise comparisons. PERMDISP (distances to centroid) in PRIMER was used to test homogeneity of multivariate dispersions among a priori groups (i.e. compost). When PERMDISP indicates that the homogeneity requirement is fulfilled, an observed significant effect can be confidently attributed to a real difference between groups and not to differences in variances between groups.

7.4. RESULTS

7.4.1. Chemical compost properties

In general, the farm composts were rich in OM (values between 43.4 ±4.3 and 75 ±3.3%/DM) (Table 7.3), due to the choice of feed-stock materials with a relatively low soil particle load and high amount of lignin-rich materials. The green waste compost is characterized by a significantly (one-way ANOVA, $p < 0.001$) higher dry matter (DM) content (i.e. 56.1 ±3.05% compared to values between 23.1 ±0.5 and 38.3 ±0.5% in the farm compost), significantly (one-way ANOVA, $p < 0.001$) higher EC value (i.e. 1254 ±53.1 µS/cm compared to values between 284 ±9.9 and 984.5 ±93.2 µS/cm in the farm compost) and a significantly (one-way ANOVA, $p < 0.02$) lower C/N ratio (13 ±2.3 compared to values between 17.4 ±1.5 and 30.1 ±1.4 in the farm compost) (Table 7.3). Within the farm composts, the Norway spruce bark compost and the willow wood chips compost had a significantly (one-way ANOVA, $p < 0.03$) lower pH (7.3 ±0.1 and 7.2 ±0.5 respectively) (Table 7.3). Large differences of Ca, Na, K and Mg concentrations between the composts were found, for both total (tot) (Table 7.4) and plant-available (plant) (Table 7.5) concentrations, and high concentrations in the feedstock materials (Table 7.4) resulted in high concentrations in the final compost. For instance, in trial 1, oak bark and Norway spruce had the highest and lowest Ca concentration respectively and this resulted in the highest and lowest Ca concentrations in finished oak bark and Norway spruce composts, for both total and plant-available concentrations. When considering compost stability, all composts, except for the willow wood chips compost and the poplar wood chips compost, had a low OUR, as values were lower than 10 mmol/kg OM/h, values typical for stable composts (Veeken et al., 2003) (Table 7.6). Ergosterol contents (Table 7.6) were significantly (one-way ANOVA, $p < 0.001$) higher for the willow wood chips compost (72 ±3.4 µg/g DM) and the external farm compost (50.8 ±4.8 µg/g DM).

Table 7.3:

P concentration, organic matter (OC) content and dry matter (DM) content in composts during the 3 composting trials and for external composts (E). Values are averages of 3 samples per compost with standard deviations in parentheses. For the external farm compost, only 1 replicate was analyzed for NO_3-N, NH_4-N, pH and EC, and only 2 replicates were measured for the other properties.

Compost and trial		$N\text{-}NO_3$ mg/l compost	$N\text{-}NH_4$ mg/l compost	$pH\text{-}H_20$ -	EC µS/cm	DM %	Total N %/DM	C/N -	Ptot mg/kg DM	OM % /DM
Oak bark	1	82.1 (3.8)	<5	8.7 (0.2)	718.8 (76.4)	29.3 (0.9)	1.7 (0.1)	19.2 (1.2)	4895 (224.3)	59.6 (0.7)
Poplar bark 1	1	81.7 (9.5)	<5	8.2 (0.1)	519.8 (33)	22.8 (0.3)	1.9 (0.1)	19.7 (1.5)	6958.2 (1277.1)	65.7 (1.7)
Norway spruce bark	1	29.1 (3)	<5	7.2 (0.5)	294.8 (19)	24.2 (0.2)	1.6 (0.1)	25.6 (0.6)	4546.8 (875)	71.9 (1.6)
75% poplar bark	2	93.5 (5.8)	7.5 (3.25)	9.4 (0.6)	901.3 (58.1)	23.1 (0.5)	2 (0.1)	19 (1.1)	3335.6 (524.7)	69.7 (2.3)
50% poplar bark	2	94.9 (11.3)	<5	9 (0.1)	984.5 (93.2)	29.5 (1.9)	1.9 (0.1)	17.4 (1.5)	3872.8 (291)	60.5 (1.3)
25% poplar bark	2	70.6 (8.8)	<5	9.1 (0.1)	983.5 (49.5)	26.6 (1)	1.8 (0.1)	18.9 (1)	4277.4 (158.7)	62.1 (1.3)
Willow wood chips	3	<5	<5	7.3 (0.1)	284.3 (9.9)	34.1 (0.6)	1.4 (0.1)	30.1 (1.4)	2403.2 (123.1)	75 (3.3)
Poplar wood chips	3	0.3 (0.6)	<5	7.8 (0.1)	311.5 (27.5)	27.8 (0.4)	1.6 (0.1)	25.2 (0.3)	2926.4 (168)	71.4 (0.5)
Poplar bark 2	3	24 (2.1)	<5	8.2 (0.1)	615.3 (38.5)	38.3 (0.5)	1.2 (0.1)	19.6 (2.1)	1514.8 (128.6)	43.4 (4.3)
Green waste	E	235.9 (4.9)	<5	7.8 (0.1)	1254.3 (53.1)	56.1 (3.1)	1.2 (0.1)	13 (2.3)	3159.5 (144.1)	28.4 (4.7)
External farm	E	78.4	<5	8.3	868	38.5 (1.1)	1.5 (0.01)	24.6 (0.1)	2063.4 (88.1)	66.2 (0.8)

Table 7.4:

Total macronutrient concentrations in the feedstock materials (F) and the composts (C). Values are averages of 3 samples per compost. Values in parentheses are standard deviations of three replicates.

Material	Type	Ca (g/kg DM compost)	Na (g/kg DM compost)	K (g/kg DM compost)	Mg (g/kg DM compost)
Poultry manure	F	20.4 (2.8)	3.06 (0.43)	30.2 (3.6)	4.8 (0.5)
Straw of grass 1	F	2.5 (0.2)	0.1 (0.02)	4.2 (0.9)	0.9 (0.2)
Oak bark	F	17.6 (2.7)	0.15 (0.01)	2.4 (0.1)	0.6 (0)
Poplar bark 1	F	14.8 (1.2)	0.16 (0.01)	3.7 (0.4)	1 (0)
Norway spruce bark	F	7 (1.4)	0.08 (0.02)	1.6 (0.3)	0.9 (0.1)
Oak bark	C	47.1 (0.7)	0.95 (0.07)	10.5 (0.6)	3.8 (0.1)
Poplar bark	C	43.7 (0.2)	0.74 (0.02)	8.5 (0.1)	4.5 (0.1)
Norway spruce	C	31.2 (3)	0.69 (0.03)	7.7 (0.2)	3.8 (0.2)
Straw of grass 2	F	2.3 (0.6)	0.22 (0.13)	12.2 (1.3)	0.5 (0.1)
Straw of clover	F	16.2 (4.2)	0.5 (0.03)	37.2 (2.7)	3.3 (1.2)
Poplar bark 2	F	23.4 (3.1)	0.33 (0.05)	4.7 (0.2)	1.2 (0)
Ensilaged maize 1	F	4 (0.9)	0.37 (0.08)	20.9 (2.4)	3.1 (0.7)
75% poplar bark	C	44.3 (4.1)	0.75 (0.03)	16.6 (0.6)	4.3 (0.3)
50% poplar bark	C	51.6 (2.7)	0.69 (0.01)	17 (0.2)	4.4 (0.1)
25% poplar bark	C	40.5 (1.8)	0.63 (0.03)	18.7 (0.8)	4.2 (0.1)
Willow wood chips	F	5.3 (0.9)	0.13 (0.01)	2.9 (0.2)	0.5 (0)
Poplar wood chips	F	5.6 (1.7)	0.08 (0)	3.7 (0.1)	0.7 (0.1)
Poplar bark 3	F	26.1 (1.4)	0.71 (0.06)	6.1 (0.8)	1.3 (0.1)
Leek	F	6.8 (2.3)	0.39 (0.05)	11.9 (0.5)	1 (0.1)
Straw of grass 3	F	2.8 (0.5)	0.1 (0.02)	1.6 (0.4)	0.5 (0.1)
Ensilaged maize 2	F	1.8 (0.2)	0.09 (0.01)	10.5 (0.4)	1.2 (0.1)
Willow wood chips	C	10.3 (0.7)	0.38 (0.01)	9.7 (0.6)	1.4 (0.1)
Poplar wood chips	C	11.7 (0.4)	0.32 (0.01)	11.4 (0.4)	1.8 (0.1)
Poplar bark	C	20.9 (1.3)	0.58 (0.03)	7.4 (0.6)	1.8 (0.2)
Green waste	C	23.7 (1.6)	1.05 (0.07)	8.1 (1)	4 (0.2)
External farm	C	49 (2.4)	0.7 (0.01)	15.6 (0.4)	5.2 (0.1)

Table 7.5:

Plant-available element concentrations in the composts. Values are averages of 3 samples per compost. Values in parentheses are standard deviations of three replicates (except for *where analyses were not replicated).

Compost	Trial	Ca (mg/l compost)	Na ((mg/l compost)	K (mg/l compost)	Mg (mg/l compost)	P (mg/l compost)
Oak bark	1	4146 (225)	206 (36)	1731 (243)	356 (23)	598 (40)
Poplar bark 1	1	4622 (289)	176 (12)	1619 (120)	510 (35)	815 (46)
Norway spruce bark	1	2195 (124)	142 (8)	1216 (78)	347 (18)	373 (24)
75% poplar bark	2	3398 (355)	94 (5)	2428 (127)	388 (30)	234 (23)
50% poplar bark	2	3332 (280)	81 (5)	2290 (65)	343 (16)	220 (16)
25% poplar bark	2	2776 (162)	70 (3)	2497 (82)	343 (15)	280 (14)
Willow wood chips	3	746 (124)	37 (4)	1042 (137)	128 (21)	116 (20)
Poplar wood chips	3	958 (41)	35 (1)	1278 (24)	186 (8)	137 (10)
Poplar bark 2	3	2565 (105)	130 (10)	1844 (111)	307 (18)	155 (10)
Green waste	E	3919 (403)	395 (33)	2939 (242)	531 (16)	340 (9)
External farm*	E	2836	108	2349	360	292

Table 7.6:
Ergosterol concentration as indicator of fungal biomass in composts (values in parentheses are standard deviations of 3 samples per compost pile), oxygen uptake rate (OUR) as an indicator of compost stability (NA: not assessed), and fresh bulk density of the tested composts.

Compost	Trial	Ergosterol (µg/g DM compost)	OUR (mmol/kg OM/h)	Bulk density (g/l compost)
Oak bark	1	8.5 (1.2)	2.5	594.2
Poplar bark 1	1	7.9 (2.2)	2.3	604.2
Norway spruce bark	1	9.2 (1.0)	1.8	556.5
75% poplar bark	2	7.0 (0.9)	3.8	522.6
50% poplar bark	2	7.7 (3.4)	3.5	538.8
25% poplar bark	2	7.1 (1.1)	3.5	575.9
Willow wood chips	3	72 (3.4)	10.1	381.0
Poplar wood chips	3	13.3 (2.3)	9.4	433.6
Poplar bark 2	3	17.8 (3.6)	4.3	593.7
Green waste	E	NA	1.8	589.1
External farm	E	50.8 (4.8)	6.6	403.0

7.4.2. Nematode community in composts

All analyzed composts contained a relatively high abundance and diversity of nematodes (Table 7.7). Total numbers of nematodes were significantly higher (one-way ANOVA, $p < 0.02$) in farm composts (range 9 ±1.1 to 44.3 ±6.6/g DM compost) compared to the green waste compost (3.3 ±0.4/g DM). The highest abundances of nematodes (>30/g DM compost) were found in the poplar wood chips compost (44.3 ±6.6/g DM), the willow wood chips compost (40 ±7.2/g DM), the compost with 25% poplar bark (34.3 ±3.8/g DM) and the poplar bark 1 compost (31 ±5.2/g DM). The fungivorous *Ditylenchus* sp. was present in all composts and was the most prominent species (42-80%), except for the compost with 75% poplar bark and the green waste compost, where, respectively, species from the bacterivorous families Panagrolaimidae (40.3 ±17.7%) and Rhabditidae (48.8 ±11.3%) were dominant. The second most common nematodes belonged to the bacterivorous family Rhabditidae (12-29%) in all composts except in the green waste compost where they were the most abundant taxon, in the external farm compost and in the poplar bark 1 compost. In the latter two composts the second most common taxa were, respectively, the putative fungivorous *Ditylenchus* sp. and Tylenchidae, and the bacterivorous Panagrolaimidae. All composts, except for the poplar wood chips compost, also contained the bacterial-feeding/predatory diplogasterids; in six out of eleven composts (i.e. the oak bark compost, the poplar bark 1 compost, the Norway spruce compost, the green waste compost and the composts with 75% and 50% poplar bark) they had a share of 10 to 20% in the total nematode community, prominent taxa being *Mononchoides composticola*, *Diplogaster* sp., *Diplogastrellus* sp. and *Diplogasteritus* sp. The species composition of the green waste compost

was significantly different (ANOSIM, p = 0.001) from the average species composition of the farm composts.

The RDA forward selection procedure, executed with the farm composts, selected 8 abiotic variables (Mg-tot, Na-tot, N-NH_4, N-NO_3, ergosterol, %DM, %OM and OUR) which together significantly explain 80% of the variation in the nematode data. The first two RDA axes (Figure 7.2) capture more than half (56%) of the total variation in the nematode data. The first RDA axis represents the most important gradient (43%) in species composition and separates the composts of trial 3 (i.e. the willow wood chips, the poplar bark 2, the external farm compost and to a lesser degree the poplar wood chips compost) from the other composts from trial 1 and 2. These composts are characterized by higher abundances of *Seinura* sp., *Ditylenchus* sp. and Tylenchidae, and have significantly ($p < 0.05$) higher values for OUR, ergosterol and %DM, and lower values for all other abiotic variables. Consistent with ergosterol as a proxy for fungal biomass, there was a significant ($p = 0.0004$) positive correlation ($R = 0.79$) between ergosterol content and the relative abundance of fungal-feeding nematodes, and a significant ($p = 0.004$) negative correlation of ergosterol content with the relative abundance of bacterial-feeding nematodes ($R = -0.87$). The composts with the highest ergosterol content also contained the highest proportion of fungal-feeders. Variation along the second RDA axis is less pronounced (13% of the variation in the nematode data). Along this axis, the oak bark and the Norway spruce compost (with higher *Diplogastrellus* sp. and Dorylaimida abundance) are separated from the poplar bark composts (i.e. the poplar bark 1 compost and the composts of trial 3), which have higher *Diplogasteritus* sp. and Rhabditidae abundance, and are characterized by significantly ($p < 0.05$) higher ammonia values and also higher values for pH, K-plant, K-tot and EC.

As illustrated in Figure 7.2, the species composition of the composts differed significantly between all three composting trials (ANOSIM, $p < 0.02$), suggesting that differences between trials were at least as large as between composts which partly differed in feedstock materials. The taxa responsible for the discrimination between the trials (SIMPER) are: Panagrolaimidae and Rhabditidae for trial 1 vs. 2; Panagrolaimidae, *Diplogaster* sp., *Ditylenchus* sp. and *Mononchoides composticola* for trial 1 vs. 3 and *Diplogaster* sp. and *M. composticola* for trial 2 vs. 3 (Figure 7.2). According to functional group composition (bacterial-feeding, fungal-feeding and bacterial-feeding/predators) trials 1 and 2 are both significantly different (ANOSIM, p = 0.001) from trial 3. Nematode assemblages did not differ significantly between different selections of bark or chips except between poplar bark and the willow wood chips (ANOSIM, p = 0.02). The oak bark, the 75% poplar bark and the green waste composts had a significantly lower (one-way ANOVA, $p < 0.05$) ΣMI compared to the other composts, while the compost of trial 3

showed significantly higher (one-way ANOVA, $p < 0.05$) ΣMI than all other composts except for the Norway spruce and the 25% poplar bark composts. The composts of trial 3 also tended to have a higher f/b ratio and CI (Table 7.7), with the highest overall f/b ratios and CI for the willow wood chips compost (i.e. 6.53 ±1.23 and 61.6 ±4.8 respectively) and the poplar bark 2 compost (i.e. 6.30 ±4.32 and 57 ±15 respectively), both with a significantly ($p \leq 0.05$) higher f/b ratio and CI than all composts of trials one and two. The 75% poplar bark compost had a significantly lower (one-way ANOVA, $p < 0.01$) f/b ratio and CI than all other composts except for the green waste compost.

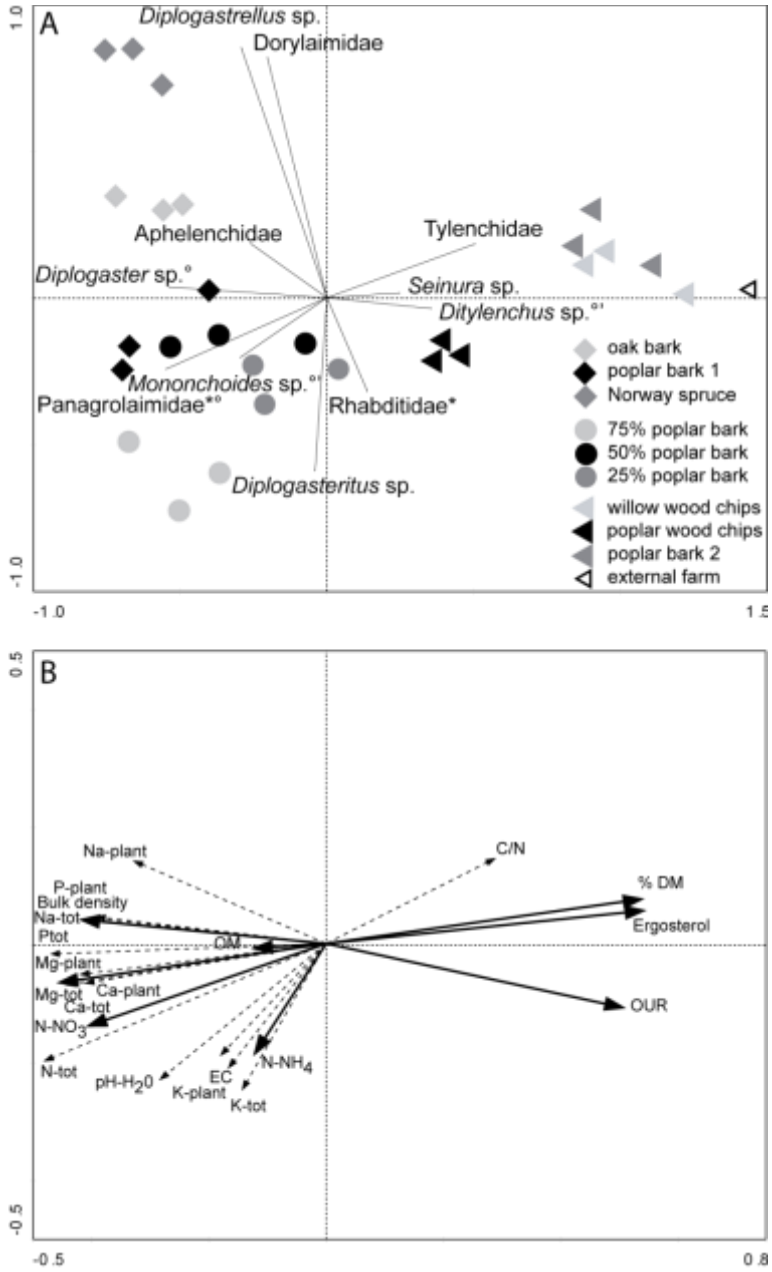


Figure 7.2:
Species-sample (A) and environmental-supplementary environmental (B) biplots of a Redundancy Analysis summarizing the effects of compost composition on nematode composition. Sample symbols show the replicates of the compost trial treatments. Abiotic parameters selected in the FS procedure (see text) are indicated with solid arrows, supplementary abiotic variables are represented by dashed arrows. For more details, see text. Results of SIMPER, indicating taxa responsible for discrimination between trials are presented with *for trial 1 and 2, °for trial 1 and 3 and ‘for trial 2 and 3 in (A).

7.4.3. Effect of compost on soil chemical properties

After 12 weeks of incubation, the main effect of compost on the soil properties was a significant increase in soil pH (one-way ANOVA, $p < 0.01$) (Table 7.8), while in blank treatments the pH slightly decreased after 12 weeks of incubation when compared to the initial pH (Table 7.1). Another overall effect of compost application was a significant (one-way ANOVA, $p < 0.01$) increase in ammonium lactate extractable K concentrations compared to blank treatments (Table 7.8). The other parameters (i.e. mineral N ($N\text{-}NO_3$ + $N\text{-}NH_4$) and ammonium lactate extractable Mg, Ca, Na and P) did not show an overall trend compared to the blank treatments, although some significant changes within incubation experiments were detected (Table 7.8). N mineralization in the compost-amended soil was significantly higher (one-way ANOVA, $p < 0.01$) compared to the blank treatments for incubation with composts of trials 1 and 2, except for the Norway spruce bark compost, but not for incubation with composts of trial 3. Both plant-available Mg and Ca concentrations significantly (one-way ANOVA, $p < 0.01$) increased in the soil for all composts except for the poplar bark composts in incubation 2 and the willow wood chips and the poplar wood chips composts of incubation 3, respectively. Significantly higher Na concentrations were only detected after incubation with green waste compost (one-way ANOVA, $p < 0.05$). For soils high in PAL, used in incubation experiment 2 (Table 7.1), no significant increases after incubation were detected (Table 7.8). High soil concentrations of plant-available Ca, Na, Mg and K (Table 7.8) after incubation with compost correspond to high plant available concentrations in the composts (Table 7.5), which were, as described above (see Section 7.3.1), the result of high concentrations in the feedstock materials. This might indicate that, from a chemical point of view, the fertilizing effect of compost on soil can be manipulated by feedstock selection.

7.4.4. Nematode community after 12 weeks of incubation

The average number of nematodes per g DM soil (Table 7.7) was higher after the compost treatments (5-23/g DM) compared to the treatments without amendment (3-6/g DM). In 6 of the 11 treatments this increase was significant (one-way ANOVA, all $p \leq 0.03$). The latter was not the case for the treatments with poplar bark 1 compost, Norway spruce bark compost, compost with 75% and 25% poplar bark and green waste compost. With only one exception (i.e. the 25% poplar bark compost) the nematode species compositions of the compost treatments were significantly different from the control treatments in the corresponding incubation experiment (PERMANOVA, $p(MC) < 0.015$ for incubation 1, $p(MC) < 0.034$ for incubation 2 and $p(MC) < 0.007$ for incubation 3). Compost treatment did not result in an unequivocal effect on the proportion

of plant-parasitic nematodes (Figure 7.3). In the first two incubations the proportions of plant-parasitic nematodes in compost treated soil remained stable or slightly increased, whereas in incubation 3 the proportion of plant-parasitic nematodes slightly decreased compared to the untreated soil. Similarly, compost amendment had a variable effect on the relative proportions of bacterial-feeding nematodes (Figure 7.3). However, species of the fungal-feeding genus *Ditylenchus* increased following all compost treatments, except for the green waste compost. This increase was significant (one-way ANOVA, $p < 0.03$) for all compost treatments, except for the treatments with 75% and 25% poplar bark composts and was on average equivalent to 1.94 specimens of *Ditylenchus* sp. per g soil DM compared to treatments without compost. Note that all *Ditylenchus* sp. are considered as fungal-feeding here, since species that are known to be epidermal cell or root hair feeders were not identified. Survival of compost nematodes appeared possible, e.g. specimens of the invertebrate predator *Seinura* sp. were found in compost and in compost-amended soil after 12 weeks of incubation, but not in the initial soil 2 nor in the control treatment. However, the diplogasterid nematodes, bacterivorous (*Diplogasteritus* sp. and *Diplogastrellus* sp.) or bacterivorous-predatory (*Mononchoides composticola* and *Diplogaster* sp.) species were most likely not retained from the compost since they were only present in very low numbers (<3%) and in very few compost-amended soils (treated with oak bark compost, Norway spruce compost and the composts with 75% and 50% poplar bark) after 12 weeks of incubation.

No clear trend was observed for the ΣMI (Table 7.7) in compost-treated soils compared to the untreated soils. The ΣMI significantly increased after treatment with poplar bark 1 compost in incubation 1 (one-way ANOVA, $p < 0.002$) but significantly decreased in treatment with willow wood chips and poplar wood chips compost in incubation 3. The f/b ratio and CI (Table 7.7) in farm compost treatments increased during all incubations compared to the treatment without compost. However, this increase was not significant ($p > 0.05$) for treatments with poplar bark compost 1, composts with 75% and 25% poplar bark and the poplar wood chips compost.

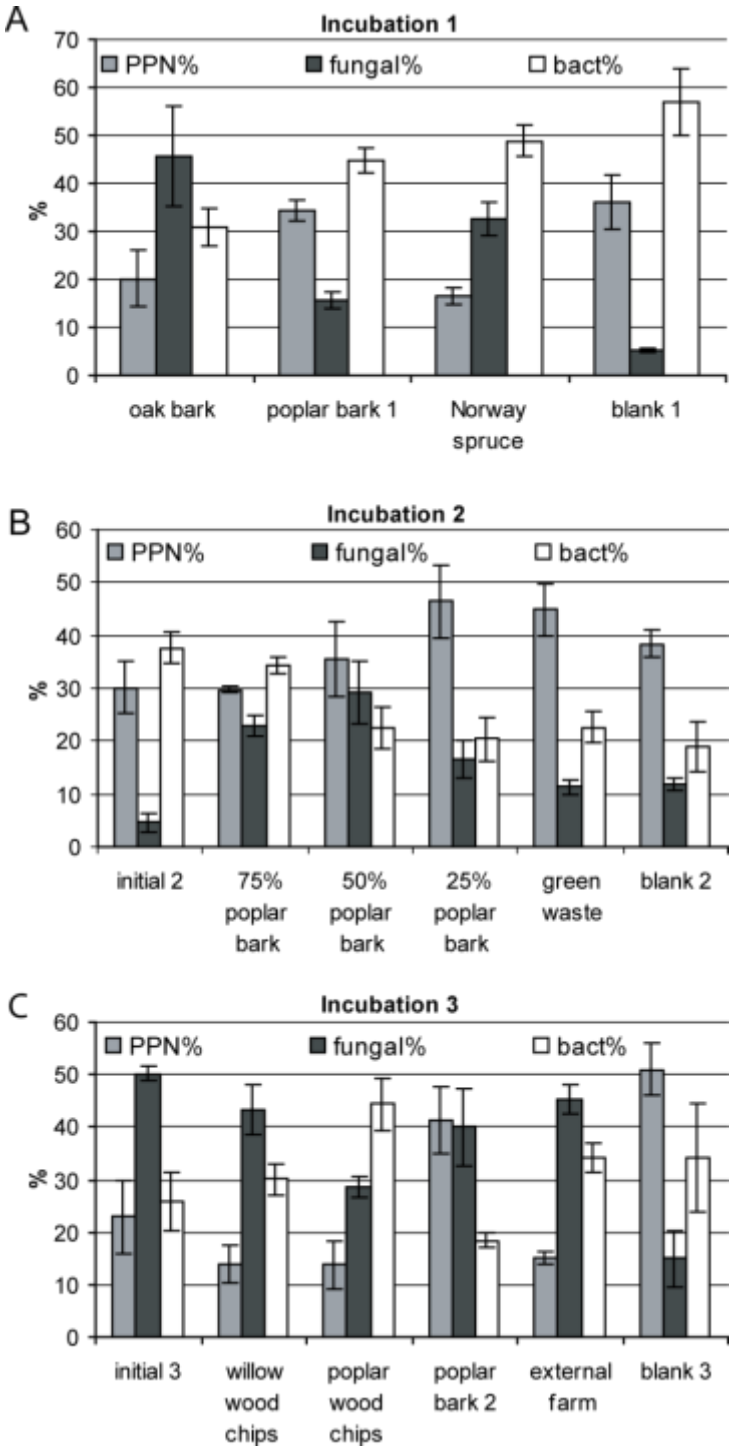


Figure 7.3:
Relative abundances of plant-parasitic nematodes (PPN%), fungal-feeding nematodes (fungal%) and bacterial-feeding nematodes (bact%) after 12 weeks of incubation. Treatments displayed per incubation experiment with blank treatments and composition of the initial soil when available.

Table 7.7:

Number of nematodes/g DM, sum Maturity Index (ΣMI), fungivorous/bacterivorous ratio (f/b), Channel Index (CI) and taxa composition of for both the used composts and the soils after incubation (mean based on 3 replicas and standard deviation between parentheses).

	COMPOST											SOIL + COMPOST															
0 % <5% 5-15% 15-30% >30%	#	MI	f/b ratio	CI	Fam. Rhabditidae	Fam. Panagrolaimidae	Fam. Aphelenchoididae	Fam. Tylenchidae	Fam. Diplogasteridae	*Ditylenchus* sp.	*Seinura* sp.	#	ΣMI25	f/b ratio	CI	Ordo Dorylaimida	Fam. Rhabditidae	Fam. Panagrolaimidae	Fam. Aphelenchoididae	Fam. Tylenchidae	Fam. Diplogasteridae	*Ditylenchus* sp.	*Seinura* sp.	Fam. Cephalobidae	Fam. Neotylenchidae	*Pratylenchus* sp.	*Rotylenchus* sp.	*Tylenchorhinchus* sp.	*Steinernema* sp.
oak bark	17 (±2.7)	1.4 (±0.04)	1.1 (±0.19)	21.4(±3.03)								8 (±0.8)	2 (±0.05)	1.5 (±0.5)	27.2 (±6.74)														
poplar bark 1	31(±5.2)	1.5 (±0.05)	2.1 (±0.32)	34.2(±3.78)								11 (±1.1)	2.1 (±0.12)	0.4 (±0.05)	8.1 (±1.07)														
Norway spruce	20 (±0.4)	1.7 (±0.04)	1.3 (±0.31)	24.6 (±4.68)								6 (±0.8)	1.7 (±0.09)	0.7 (±0.12)	14.3 (±2.14)														
blanco I												6 (±0.6)	1.6 (±0.02)	0.09 (±0.02)	2.3 (±0.39)														
75% poplar bark	25 (±2.9)	1.2 (±0.07)	0.3 (±0.13)	7.6 (±2.79)								4 (±1.3)	4.3 (±1.05)	0.7 (±0.09)	14.4 (±1.69)														
50% poplar bark	19 (±2)	1.6 (±0.05)	1.7 (±0.32)	29.4 (±3.87)								8 (±1.5)	6 (±1.23)	1.3 (±0.38)	24.7 (±5.47)														
25% poplar bark	34 (±3.8)	1.6 (±0.05)	2 (±0.49)	33.2 (±5.69)								5 (±0.2)	3.5 (±0.14)	0.8 (±0.14)	16.8 (±2.35)														
green waste	3 (±0.4)	1.4 (±0.1)	0.7 (±0.31)	14.6 (±5.66)								5 (±0.9)	7.8 (±1.27	0.6 (±0.11)	11.2 (±2.33)														
blanco II												5 (±0.6)	1.3(±0.27)	0.5 (±0.12)	13.9 (±2.13)														
willow wood chips	40 (±7.2)	1.9 (±0.02)	6.5 (±1.23)	61.6 (±4.8)								8 (±0.5)	1.7 (±0.07)	1.5 (±0.28)	26.9 (±3.91)														
poplar wood chips	44 (±6.6)	1.7 (±0.07)	2.9 (±1.01)	41.4 (±8.75)								23 (±1.8)	1.4 (±0.06)	0.7 (±0.11)	14 (±2.07)														
poplar bark 2	9 (±1.1)	1.9 (±0.1)	6.3 (±4.32)	57 (±14.91)								7 (±0.3)	1.8 (±0.04)	2.2 (±0.26)	35 (±5.59)														
farm compost	18 (±1)	1.8 (±0.03)	3.1 (±0.59)	43.7 (±4.51)								14 (±3)	1.9 (±0.04)	1.3 (±0.12)	24.9 (±1.74)														
blanco III												3 (±0.4)	2 (±0.06)	0.5 (±0.37)	11 (±7.15)														

Table 7.8:

Soil properties (mean based on 3 replica's and standard deviation in parentheses), of the soils after 12 weeks of incubation (Nmin = N-NO3 + N-NH4). Treatments were compared with one-way ANOVA for each incubation trial separately. Means that are significantly different are denoted with different letters (one-way ANOVA and subsequent post hoc Tukey HSD tests on log-transformed data).

Compost	Incubation exp.	pH-KCl	Nmin (mg/kg DM)	P (mg/100g DM)	K (mg/100g DM)	Mg (mg/100g DM)	Mn (mg/100g DM)	Ca (mg/100g DM)	Na (mg/100g DM)
Oak bark	1	5.81 (0.06) c	61.7 (4.4) bc	22.3 (1.2) b	23.3 (0.6) c	11.2 (0.7) b	12.6 (0.7) b	85.4 (5.8) b	3.3 (1.1) a
Poplar bark 1	1	5.92 (0.06) c	54.1 (3.4) c	22.5 (1.0) b	21.0 (0.7) b	10.8 (1.1) b	11.3 (0.4) a	82.7 (6.4) b	1.9 (0.6) a
Norway spruce bark	1	5.49 (0.10) b	50.5 (4.1) ab	20.5 (1.3) b	23.4 (0.9) c	11.3 (0.3) b	14.1 (0.6) c	76.1 (5.3) b	3.4 (1.1) a
Blank 1	1	5.14 (0.01) a	42.3 (5.0) a	16.5 (1.3) a	13.7 (0.5) a	7.5 (0.3) a	10.8 (0.5) a	59.0 (1.2) a	1.7 (1.0) a
75% poplar bark	2	6.40 (0.01) b	48.1 (4.3) b	50.4 (3.3) a	57.0 (0.7) c	19.1 (0.1) a	8.5 (0.3) a	113.5 (0.7) ab	2.9 (0.4) a
50% poplar bark	2	6.46 (0.03) b	45.5 (11.6) b	50.1 (2.7) a	58.2 (0.8) c	19.8 (0.7) a	8.5 (0.4) a	118.9 (4.6) bc	2.7 (0.2) a
25% poplar bark	2	6.40 (0.04) b	54.1 (2.4) b	51.3 (1.9) a	59.6 (2.7) c	19.8 (0.8) a	8.6 (0.2) a	111.7 (4.5) ab	2.6 (0.3) a
Green waste	2	6.46 (0.09) b	56.9 (4.9) b	49.1 (8.0) a	53.1 (1.3) b	23.3 (0.4) b	9.0 (1.4) a	125.8 (2.4) c	6.6 (0.3) b
Blank 2	2	6.15 (0.01) a	30.0 (0.7) a	46.9 (4.0) a	30.2 (1.4) a	19.0 (0.6) a	8.5 (0.4) a	103.9 (2.7) a	2.1 (0.3) a
Willow wood chips	3	5.37 (0.05) b	93.2 (22.2) a	12.2 (0.6) b	36.6 (2.4) b	7.5 (0.5) b	2.2 (0.1) b	54.3 (4.4) a	7.4 (1.3) a
Poplar wood chips	3	5.36 (0.04) b	102.1 (17.1) a	12.0 (0.6) b	35.6 (1.6) b	7.7 (0.2) b	2.2 (0.1) b	53.7 (1.0) a	6.3 (0.5) a
Poplar bark 2	3	5.71 (0.04) c	117.1 (17.6) a	11.7 (0.4) a	34.2 (0.8) b	8.5 (1.0) b	2.3 (0.2) b	72.6 (8.2) b	6.8 (1.1) a
External farm	3	6.30 (0.01) d	107.3 (14.7) a	13.3 (0.2) b	47.6 (1.8) c	11.2 (0.5) c	3.0 (0.2) c	88.7 (4.5) c	5.8 (0.7) a
Blank 3	3	4.91 (0.01) a	82.8 (6.6) a	10.0 (0.8) a	23.7 (1.9) a	4.9 (0.2) a	1.5 (0.1) a	45.2 (2.0) a	5.9 (1.0) a

7.5. DISCUSSION

7.5.1. Omnipresence of nematodes in composts and their potential to reflect compost status

In all composts in this study, relatively high abundances of nematodes were found (on average 26 nematodes/g DM), the only exception being the green waste compost with only 3 ±0.4 nematodes/g DM, whereas for the farm compost densities from approximately 10-50 nematodes/g DM were found, which tends to be higher than previously described abundances for farm compost (9.3 ±1 nematodes/g DM; Chapter 2), and which meets the standard of 20-100 beneficial nematodes/g DM of the Soil Foodweb Inc. company (except for the poplar bark 2 compost). These densities are comparable to nematode densities commonly found in soil under agricultural use, where between 1 and 250 nematodes/g DM are found (Freckman and Ettema 1993; Hu and Qi 2010a; Liang et al. 2005; Viketoft et al. 2011). However, these numbers all include also plant-parasitic nematodes while in compost only free living nematodes are found. The taxon composition for all analyzed composts in this study was remarkably similar to that described in Chapter 2 with *Mononchoides composticola*, *Diploscapter coronatus*, *Halicephalobus gingivalis*, *Ditylenchus* sp., *Diplogaster* sp., *Diplogastrellus* sp. and *Diplogasteritus* sp. being virtually omnipresent. The trophic group composition was also very similar: 40-85% fungal-feeding nematodes, 13-40% bacterial-feeding nematodes, and 5-20% bacterial-feeders/predators in the mature compost of this study, compared to, respectively, ±80%, ±10% and ±5% in that of Chapter 2. This similarity in density and tropic structure might be explained by the selective nature of a fast changing (succession of composting phases) though very specific compost habitat (in terms of organic materials, moisture levels and temperatures). The family Tylenchidae was recorded for the first time in compost in the present study, i.e. the genus *Filenchus* in the willow wood chips, the poplar bark 2 and the external farm compost. Generally, Tylenchidae are considered associates of algae, mosses, lichens and plant roots (Siddiqi 2000) and only recently *Filenchus* was shown to be capable of feeding on fungi (Okada et al. 2005). Current records confirm that at least some Tylenchidae (basal Tylenchomorpha/plant-parasitic nematodes) are independent of plants (Bert et al. 2011). When considering the nematode community structure, the differences between trials were larger than differences within trials (see also RDA plot in Figure 7.2). Hence, the trial effect is larger than the effect of the chosen feedstock material. This contrasts with the chemical compost properties where substantial differences were found between composts with different feedstock materials, in agreement with previous studies (Francou et al. 2005; Grigatti et al. 2011). For the green waste compost

significantly lower nematode abundances and different species were found. This might be explained by the lack of careful selection of the feedstock materials (in contrast with farm composts) or by repeated high temperature peaks and/or low moisture levels, but in the absence of regular measurements during composting, this explanation remains speculative. Nevertheless, although only one green waste compost was included in this study, the compost method appears to have a considerable effect on the nematode community. In contrast, the exact types and proportions of feedstock materials had only a moderate effect on the nematode community composition and abundance.

Results of the farm composts indicate that those composts that exhibit a species composition or distribution of functional groups that differs to some extent from the typical compost community as described above, also showed deviating values of one or more of the abiotic parameters. For example: for both the willow wood chips compost and the poplar wood chips compost values of oxygen uptake rate (OUR) were rather high (>8), indicating relatively low compost maturity (Grigatti et al. 2011; Kalamdhad et al. 2008), and the NO_3/NH_4 ratio was very low (<1) indicating instability (Bernal et al. 1998). And, remarkably, the proportion of nematode feeding groups in these composts deviated from that in most other composts, with a lack or very low numbers of bacterial-feeders/predators like *Mononchoides composticola* and/or *Diplogaster* sp. and an unusually high proportion of fungal-feeding *Ditylenchus* sp. (75-85%). The latter agrees with the high ergosterol concentrations in these composts (Figure 7.2). The time span of the composting process may well be important here, since trial 3 only lasted 24 weeks and included no storage period, but resulted, based on a high OUR (>8) and low (<1) NO_3/NH_4 ratio, in two out of three immature composts. The external farm compost also showed high ergosterol concentration along with a high proportion of fungal-feeding nematodes (65% on average) but no signs of instability. When nematode communities between composts based on used feedstock were compared (see Section 7.3.2), only a significant difference between the poplar bark based composts and the willow wood chips compost was detected. However, as mentioned above, the willow wood chips compost is not completely mature, which may explain the difference in nematode communities rather than this being an effect of the chosen feedstock material. Thus, despite the largely different feedstock materials and proportions and the clear differences in chemical properties (Section 7.3.1), biologically very similar composts were produced, the only major difference in nematode assemblages being between immature (i.e. willow wood chips and poplar wood chips) and mature composts. In conclusion, since the nematode community structure appears to be linked with the ergosterol content and the abiotic parameters (C/N ratio, DM and OUR) of the composts (Figure 7.2), nematodes can potentially be

valuable potential candidates as indicators of compost status regarding stability and maturity, but seem not to be related to the type and proportions of the feedstock materials. The overall quality of the feedstock materials (i.e. accurate selection or merely unselected waste material) combined with the composting process (i.e. for example time span of composting phases, height and duration of temperature peaks, approximation of the proportion of 60% brown and 40% green material) appears to be more crucial for nematode populations in the final product than the proportion or specific mix of the feedstock material.

7.5.2. Effects of compost addition: compost nematodes vs. existent soil-nematode communities

In terms of available macro-nutrients, compost treatments did not significantly increase mineral N or plant-available P (Table 7.8) for soils initially high in mineral N and PAL (Table 7.1), i.e., in incubation trials 3 and 2, respectively. Hence, our results indicate that the distinctiveness of the compost effect on the soil properties (Table 7.8) depends on the initial elemental or nutrient concentrations in the soil (Table 7.1). Regarding soil chemical properties, the main effect of incubation with compost was a significant increase in soil pH. Organic amendments have previously been reported to serve as a buffer against acidification (Koopmans et al., 2007). An increased pH in soil is known to stimulate the growth of bacteria and Actinomycetes (see also D'Hose et al. 2010) which favors bacterial-feeding nematodes and causes accelerated degradation of soil organic matter and release of nutrients such as nitrogen and phosphorus, while numbers of fungal-feeding nematodes decreased under conditions of increasing pH (Korthals et al. 1996). In this study however, compost addition generally resulted in an increase of the density of total nematodes and of fungal-feeding nematodes in soil, while the effects on bacterial-feeding nematodes were inconsistent. There is considerable though often conflicting evidence in recent literature on changes in the abundance and structure of the soil nematode community due to the addition of organic amendments (for a review see, Thoden et al. 2011). A marked increase in nematode population density after compost application was also found by Kimpinski et al. (2003), Yao et al. (2006), Langat et al. (2008), and Liang et al. (2009), but not by Garcia-Alvarez et al. (2004), Yeates et al. (2006) and Hu and Qi (2010a). Organic amendments were generally found to increase the density of bacterial-feeding nematodes (Bulluck et al. 2002; Hu and Cao, 2008; Hu and Qi 2010b; Porazinska et al. 1999), although Renčo et al. (2010) found that, as in this study, some composts suppressed bacterial-feeding nematodes while others increased them. The increase in bacterial-feeding nematodes, which was only rarely and inconsistently observed in this study, is usually attributed to the corresponding increase in bacterial populations (Bongers and Ferris 1999; Bulluck and Ristaino 2002; Ferris et al. 1997;

Forge et al. 2003). In our experiment, the most distinct increase in bacterial-feeding nematodes, compared to blank treatment, was observed following 75% poplar bark compost addition. This increase might indicate a stimulatory effect of compost addition (by stimulating bacterial populations) or might be explained by the actual addition of nematodes with the compost, since this compost also contained the highest relative abundance of bacterial-feeding nematodes. In line with the latter explanation, we found that the most pronounced effect of the composts (green waste excluded) on the existent soil nematode community was the increase in fungivorous nematodes. Such an increase was also described by Bulluck et al. (2002) and Leroy et al. (2009), while the reverse was described by Renčo et al. (2010). However, the presence of nematodes in compost amendments is often overlooked. To the best of our knowledge, this incubation experiment is the first where the nematode population of both the composts and the amended soils were analyzed. Since only a single genus, *Ditylenchus*, was responsible for this increase it is plausible that an increased number of fungivorous nematodes is only the result of inoculation with compost nematodes and not of any stimulatory effect of the compost. Taking into account the dilution effect, assuming nematode survival after 12 weeks, and using the control treatment soil as a reference, a hypothetical calculation indeed shows that the increased abundance of fungal-feeding nematodes may at least partly be a direct effect of inoculation. This was the case for *Ditylenchus* specimens in the compost with 25% poplar bark: an average of 40.0 ±0.03 individuals per 100 g dry weight were added with the compost, while after 12 weeks of incubation only an extra 12.0 ±0.2 individuals per 100 g dry weight soil were found compared to the untreated soil. This has critical implications for the interpretation of former studies on the effect of compost on the soil nematode community; in the latter, compost was treated as a black box devoid of nematodes. For example, Kimpinski et al. (2003) described a difference of 1560 nematodes/kg dry soil between compost amended and control plots. Theoretically, nematodes already present in the compost could have been completely responsible for this difference: if we assume a comparable nematode density in the applied compost as found in our study (on average 26/g dry weight), and taking into account the doses of compost added (i.e. 50 t/ha in our study and 16 t/ha in Kimpinski et al. (2003)), more than 8000 nematodes/kg dry soil were added with the compost, which greatly exceeds the observed increase. To test whether compost nematodes effectively survive and reproduce in soil and to prove that the observed increase is not only caused by the added effect of the compost, one might try to use appropriate molecular tracer methods like microsatellites (Selkoe and Toonen 2006), though it remains a highly complex transfer to prove. In the case of *Seinura* sp., an invertebrate predator which was not present in the initial and blank soil, inoculation with compost was successful for at least 12

weeks. This was probably not the case for the bacterial-feeder/predators since they were added in high numbers but could not be recovered from the soil after 12 weeks of incubation. This seriously hampers their biocontrol potentials as suggested in Chapter 3 (part 2) and by Bilgrami (2008).

In conclusion, the most important short-term effect of the used farm composts on the soil was an increase of total and fungal-feeding nematodes irrespective of the used feedstock materials used in the compost. However, we have demonstrated that such results should be interpreted with caution, i.e. that the addition effect of compost nematodes should not be neglected. In contrast to the nematode community, the soil chemical properties after incubation could be linked to the properties of the used feedstock materials. Future research should assess whether the populations added with compost actively survive and reproduce in soil, and whether the beneficial effects of compost amendment on soil chemical properties affects nematode communities in the longer run.

Acknowledgements

Hanne Steel acknowledges the Foundation for Scientific Research, Flanders (FWO-Flanders, Belgium) for providing a PhD grant as aspirant. We are grateful to the technical staff and the lab technicians at ILVO for their contribution to this research.

7.6 REFERENCES

Akhtar, M., Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. Bioresour. Technol. 74, 35-47.

Bailey, K.L., Lazarovits, G., 2003. Suppressing soil-borne diseases with residue management and organic amendments. Soil Till. Res. 72, 169-180.

Bernal, M.P., Paredes, C., Sanchez-Monedero, M.A., Cegarra, J., 1998. Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresour. Technol. 63, 91-99.

Bert, W., Karssen, G., Helder, J., 2011. Phylogeny and evolution of nematodes. In: Jones, J., Gheysen, G., Fenoll, C. (Eds.), COST Action 872 Exploiting Genomics to Understand Plant-Nematode Interactions. Springer, Dorderecht, pp. 45-59.

Bilgrami, A.L., 2008. Biological control potentials of predatory nematodes. In: Ciancio, A., Mukerji, K.G. (Eds.), Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer, pp. 3-28.

Boag, B., Yeates, G.W., 1998. Soil nematode biodiversity in terrestrial ecosystems. Biodivers. Conserv. 7, 617-630.

Bongers, T., 1990. The maturity index - an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19. Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. 14, 224-228.

Bongers, T., 1999. The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. Plant Soil 212, 13-22.

Brussaard, L., Kuyper, T.W., Didden, W.A.M., De Goede, R., Bloem, J., 2004. Biological soil quality from biomass to biodiversity - importance and resilience to management stress and disturbance. In: Schjonning, P., Elmholt, S., Christensen, B.T. (Eds.), Managing Soil Quality: Challenges in Modern Agriculture. CAB International, Oxfordshire, pp. 139-161.

Bulluck, L.R., Barker, K.R., Ristaino, J.B., 2002. Influences of organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomatoes. Appl. Soil Ecol. 21, 233-250.

Bulluck, L.R., Ristaino, J.B., 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. Phytopathology 92, 181-189.

Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd edition. Plymouth, PRIMER-E. Cogger, C.G., 2005. Potential compost benefits for restoration of soils disturbed by urban development. Compost Sci. Util. 13, 243-251.

D'Hose, T., Cougnon, M., De Vliegher, A., Van Bockstaele, E., Reheul, D., 2010. Influence of farm compost on soil quality and crop yields. In: Proceedings International Conference on Soil Fertility and Productivity, Two Features to be Distinguished-differences of Efficiency of Soils for Land Uses, Expenditures and Returns.

D'Hose, T., Cougnon, M., De Vliegher, A., Willekens, K., Van Bockstaele, E., Reheul, D., 2012. Farm compost application: effects on crop performance. Compost Sci. Util. 20, 49-56.

De Cauwer, B., Van den Berge, K., Cougnon, M., Bulcke, R., Reheul, D., 2010. Weed seedbank responses to 12 years of applications of composts, animal slurries or mineral fertilisers. Weed Res. 50, 425-435.

De Cauwer, B., D'Hose, T., Cougnon, M., Leroy, B., Bulcke, R., Reheul, D., 2011. Impact of the quality of organic amendments on size and composition of the weed seed bank. Weed Res. 51, 250-260.

De Grisse, A.T., 1969. Redescription ou modifications de quelques techniques utilisées dans l' étude des nématodes phytoparasitaires. Meded. Rijsfak. Landbouwet. Gent 34, 351-369.

Egnèr, H., Riehm, H., Domingo, W.R., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. Kung. Lantbrukshögsk. Ann. 26, 199-215.

Ferris, H., Venette, R.C., Lau, S.S., 1997. Population energetics of bacterial-feeding nematodes: carbon and nitrogen budgets. Soil Biol. Biochem. 29, 1183-1194.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Forge, T.A., Hogue, E., Neilsen, G., Neilsen, D., 2003. Effects of organic mulches on soil microfauna in the root zone of apple: implications for nutrient fluxes and functional diversity of the soil food web. Appl. Soil Ecol. 22, 39-54.

Francou, U., Poitrenaud, M., Houot, S., 2005. Stabilization of organic matter during composting: influence of process and feedstocks. Compost Sci. Util. 13, 72-83.

Freckman, D.W., Ettema, C.H., 1993. Assessing nematode communities in agro-ecosystems of varying human intervention. Agric. Ecosyst. Environ. 45, 239-261.

Garcia-Alvarez, A., Arias, M., ez-Rojo, M.A., Bello, A., 2004. Effect of agricultural management on soil nematode trophic structure in a Mediterranean cereal system. Appl. Soil Ecol. 27, 197-210.

Gong, P., Guan, X., Witter, E., 2001. A rapid method to extract ergosterol from soil by physical disruption. Appl. Soil Ecol. 17, 285-289.

Goralczyk, K., 1998. Nematodes in a coastal dune succession: indicators of soil properties? Appl. Soil Ecol. 9, 465-469.

Grigatti, M., Cavani, L., Ciavatta, C., 2011. The evaluation of stability during the composting of different starting materials: comparison of chemical and biological parameters. Chemosphere 83, 41-48.

Hooper, D.J., 1986. Extraction of free-living stages from soil. In: Southey, J.F. (Ed.), Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationary Office, London, pp. 5-30.

Hu, C., Cao, Z.P., 2008. Nematode community structure under compost and chemical fertilizer management practice, in the North China Plain. Exp. Agric. 44, 485-496.

Hu, C., Qi, Y.C., 2010a. Effect of compost and chemical fertilizer on soil nematode community in a Chinese maize field. Eur. J. Soil Biol. 46, 230-236.

Hu, C., Qi, Y.C., 2010b. Abundance and diversity of soil nematodes as influenced by different types of organic manure. Helminthologia 47, 58-66.

Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi, and their nematode grazers - effects on nutrient cycling and plant-growth. Ecol. Monogr. 55, 119-140.

Jongman, R.H.G., ter Braak, C.J.F., Van Tongeren, O.F.R., 1995. Data analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, United Kingdom.

Kalamdhad, A.S., Pasha, M., Kazmi, A.A., 2008. Stability evaluation of compost by respiration techniques in a rotary drum composter. Resour. Conserv. Recy. 52, 829-834.

Kimpinski, J., Gallant, C.E., Henry, R., MacLeod, J.A., Sanderson, J.B., Sturz, A.V., 2003. Effect of compost and manure soil amendments on nematodes and on yields of potato and barley: a 7-year study. J. Nematol. 35, 289-293.

Koopmans, C., Bokhorst, J., ter Berg, C., van Eekeren, N., 2007. Bodemsignalen: praktijkgids voor een vruchtbare bodem, pp. 1-96.

Korthals, G.W., Alexiev, A.D., Lexmond, T.M., Kammenga, J.E., Bongers, T., 1996. Long-term effects of copper and pH on the nematode community in an agroecosystem. Environ. Toxicol. Chem. 15, 979-985.

Kuo, S., Ortiz-Escobar, M.E., Hue, N.V., Hummel, R.L., 2004. Composting and compost utilization for agronomic and container crops. Recent Res. Dev. Environ. Biol. 1, 451-513.

Langat, J.K., Kimenju, J.W., Mutua, G.K., Muiru, W.M., Otieno, W., 2008. Response of free-living nematodes to treatments targeting plant parasitic nematodes in carnation. Asian J. Plant Sci. 7, 467-472.

Leroy, B.L.M., De Sutter, N., Ferris, H., Moens, M., Reheul, D., 2009. Short-term nematode population dynamics as influenced by the quality of exogenous organic matter. Nematology 11, 23-38.

Leroy, B.L.M.M., Bommele, L., Reheul, D., Moens, M., De Neve, S., 2007. The application of vegetable, fruit and garden waste (VFG) compost in addition to cattle slurry in a silage maize monoculture: effects on soil fauna and yield. Eur. J. Soil Biol. 43, 91-100.

Liang, W., Lou, Y., Li, Q., Zhong, S., Zhang, X., Wang, J., 2009. Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China. Soil Biol. Biochem. 41, 883-890.

Liang, W.J., Lavian, I., Pen-Mouratov, S., Steinberger, Y., 2005. Diversity and dynamics of soil free-living nematode populations in a Mediterranean agroecosystem. Pedosphere 15, 204-215.

Nahar, M.S., Grewal, P.S., Miller, S.A., Stinner, D., Stinner, B.R., Kleinhenz, M.D., Wszelaki, A., Doohan, D., 2006. Differential effects of raw and composted manure on nematode community, and its indicative value for soil microbial, physical and chemical properties. Appl. Soil Ecol. 34, 140-151.

Naidu, R., Megharaj, M., Owens, G., 2004. Recyclable urban and industrial waste - benefits and problems in agricultural use. In: Schjonning, P., Elmholt, S., Christensen, B.T. (Eds.), Managing Soil Quality: Challenges in Modern Agriculture. CAB International, Oxfordshire, pp. 219-237.

Oka, Y., 2010. Mechanisms of nematode suppression by organic soil amendments - a review. Appl. Soil Ecol. 44, 101-115.

Okada, H., Harada, H., Kadota, I., 2005. Fungal-feeding habits of six nematode isolates in the genus Filenchus. Soil Biol. Biochem. 37, 1113-1120.

Pfotzer, G.H., Schüler, C., 1997. Effects of different compost amendments on soil biotic and faunal feeding activity in an organic farming system. Biol. Agric. Hortic. 15, 177-183.

Porazinska, D.L., Duncan, L.W., McSorley, R., Graham, J.H., 1999. Nematode communities as indicators of status and processes of a soil ecosystem influenced by agricultural management practices. Appl. Soil Ecol. 13, 69-86.

Renčo, M., Sasanelli, N., D'Addabbo, T., Papajova, I., 2010. Soil nematode community changes associated with compost amendments. Nematology 12, 681-692.

Reuveni, R., Raviv, M., Krasnovsky, A., Freiman, L., Medina, S., Bar, A., Orion, D., 2002. Compost induces protection against *Fusarium oxysporum* in sweet basil. Crop Prot. 21, 583-587.

Ruess, L., 2003. Nematode soil faunal analysis of decomposition pathways in different ecosystems. Nematology 5, 179-181.

Seinhorst, J.W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12, 178.

Selkoe, K.A., Toonen, R.J., 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol. Lett. 9, 615-629.

Siddiqi, M.R., 2000. Tylenchida Parasites of Plants and Insects, 2nd edition. CABI Publishing, Wallingford, UK, 864 pp.

ter Braak, C.J.F., Smilauer, P., 1998. Canoco Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer Power, Ithaca, NY.

Termorshuizen, A.J., Moolenaar, S.W., Veeken, A.H.M., Blok, W.J., 2004. The value of compost. Rev. Environ. Sci. Biotechnol. 3, 343-347.

Thoden, T.C., Korthals, G.W., Termorshuizen, A.J., 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematology 13, 133-153.

Veeken, A.H.M., de Wilde, V., Hamelers, H.V.M., Moolenaar, S.W., Postma, R., 2003. Oxitop Measuring System for Standardised Determination of the Respiration Rate and N-mineralisation Rate of Organic Matter in Waste Material, Compost and Soil. Wageningen University and Nutrient Management Institute.

Viketoft, M., Sohlenius, B., Bostrom, S., Palmborg, C., Bengtsson, J., Berg, M.P., Huss-Danell, K., 2011. Temporal dynamics of soil nematode communities in a grassland plant diversity experiment. Soil Biol. Biochem. 43, 1063-1070.

Yao, S., Merwin, I.A., Abawi, G.S., Thies, J.E., 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. Soil Biol. Biochem. 38, 587-599.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera - an outline for soil ecologists. J. Nematol. 25, 315-331.

Yeates, G.W., Speir, T.W., Taylor, M.D., Clucas, L., Van Schaik, A.P., 2006. Nematode responses to biosolids incorporation in five soil types. Biol. Fertil. Soils 42, 550-555.

Zorpas, A.A., 2009. Compost evaluation and utilization. In: Pereira, C.J., Bolin, J.L. (Eds.), Composting: Processing, Materials and Approaches. Nova Science Publishers Inc., New York, pp. 31-68.

Zvomuya, F., Larney, F.J., DeMaere, P.R., Olson, A.F., 2008. Organic amendment effects on crop productivity and nutrient uptake on reclaimed natural gas wellsites. Nutr. Cycl. Agroecosys. 80, 223-232.

8. General Discussion

In the framework of this PhD project, the diversity, structure and succession of the nematode community during composting were studied for the first time. In total 9, complete composting processes were examined: *i.e.* 5 farm composting processes (using the Controlled Microbial Composting method), 1 industrial green waste process, and 3 small-scale processes in compost barrels (each in three replicates, cfr. treatments in chapter 5). In addition, finished composts resulting from seven other compost processes were also analyzed.

Given their important relation with nematodes and because we aimed to compare their use as indicators of compost maturity with that of nematodes, the microbial community of 4 out of 5 farm composting processes and the industrial green waste process were examined, this based on phospholipid fatty acid (PLFA) extractions.

Current study is the first comprehensive study on the succession of nematode communities in compost and this resulted in several new findings and insights. First, repeatable patterns of nematode succession were observed in the monitored composting processes (Chapter 2, 4, 6). Second, the nematode community analyses proved to be more efficient in tracking the different composting stages compared to microbial PLFA patterns. Third, experimental evidence confirms the potential of soil-dwelling nematodes to disperse over a relatively long distance in a very short time and indicates insects as vector (Chapter 5). Additionally, we demonstrated that previously reported short-term effects of compost amendments on the soil nematode community may to a large extent have been caused by the addition of nematodes already present in the compost (Chapter 7).

8.1 MICROBIAL COMMUNITIES AS INDICATORS OF COMPOST MATURITY: METHODOLOGICAL CONSIDERATIONS

Unlike the nematode community, the succession of the microbial community during composting has been more extensively studied and relatively well documented. This may be attributed to their role as main decomposers which directly contribute to the degradation process. However, only few studies have described the diversity of fungi during an entire composting process (Ryckeboer et al. 2003).

Compared to the nematode community succession, the changes in the microbial community as based on our PLFA analyses were mostly concentrated in the first month of composting and were less pronounced and not unequivocal among examined processes (Chapter 4 *vs.* 6). No distinct changes in the major microbial functional groups (gram-positive, gram-negative,

actinomycetes and fungi) related with compost maturity were found, and especially the absence of a clear pattern in the later compost stages hampers the use of the microbial community as a bio-indicator of compost maturity. There is only one exception: the abundance of actinomycetes generally increases during the process, as confirmed by our results, and the change of their assemblage composition may be a possible indicator of maturity (Steger et al. 2007a, 2007b). Although PLFA analyses are generally regarded as a sensitive and reliable method to quantitatively asses the changes in biomass in the major groups of microorganisms (Zeller 1999; Frostegård et al. 2011), the assumed turnover rate of PLFAs and the specificity of markers for any particular group are still subject to intense discussion (e.g. Kaiser et al. 2010; Ruess and Chamberlain 2010). The issue of specificity of biomarkers is especially problematic for fungal markers (Joergensen and Wichern 2008, Ruess and Chamberlain 2010), and our results (Chapter 6) indicate that it is indeed for this group that doubtful PLFA-based quantifications in compost can be expected. The fungal diversity is known to increase with time of composting (Ryckeboer et al. 2003) and it is not impossible that some forms or species of fungi, which are especially important at the end of the process, are not detected with current PLFA techniques. Further screening of the lipid pattern of soil decomposers is necessary to reveal unique fatty acids within food web members. Furthermore, retaining the neutral lipid fatty acids (NLFAs) next to the PLFAs, could be a useful tool to indicate trophic links in the food web, in addition to information on microbial biomass and community structure inferred from the PLFAs. Trophic links may be unraveled through dietary routing, *i.e.* based on the assimilation of marker fatty acids from phospholipids in microbial food sources into neutral lipids of decomposer animals (Ruess 2005, Ruess and Chamberlain 2010).

An alternative possibility to analyze the microbial community using PLFA might be fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and ARISA (Automated Ribosomal Intergenic Spacer Analysis). DGGE of ribosomal genes has for instance successfully been used to identify the main microbial community succession patterns (*i.e.* bacteria and fungi) in compost (Steger et al. 2007a, 2007b; Novinscak et al. 2009). Furthermore, DGGE of functional genes can simultaneously provide information about microbial function and diversity (Tabatabaei et al. 2009). Even so, fingerprinting techniques still cope with problems of over- but mainly underestimation of diversity (Xu 2012). At this moment, several next generation DNA sequencing technologies are becoming widely available and metagenetics and metagenomics approaches of environmental samples are becoming an important tool for understanding taxonomic, functional and ecological biodiversity (Shokralla et al. 2012). Environmental sequencing using the metagenomics approach analyses all genes from all members of the

sampled community and thus will probably provide the most complete picture including rare and unknown diversity.

8.2 NEMATODE SUCCESSION IN COMPOST, A NEWLY EXPLORED HABITAT

According to current knowledge, of all mesofauna found in compost (Chapter 1), only nematodes have consistently been found in every compost pile that has hitherto been investigated, and they are effectively omnipresent during all stages of the composting process (Chapter 2, 4, 5, 6, 7), except sometimes during the heat peaks.

Remarkably similar major shifts in nematode assemblage composition occurred in all composting processes in the current study, except in the industrial green waste compost. This succession of nematodes is associated with major shifts in life strategies and feeding behavior (Figure 8.1). At the beginning of the process (thermophilic phase), immediately after the heat peak, the nematode population primarily consisted of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae and Diplogasteridae), supplemented with fewer bacterial-feeding general opportunists (cp-2) (Cephalobidae) and/or with the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, during the cooling and maturation stages, first the bacterial-feeding/predator enrichment opportunistic nematodes (mainly *Mononchoides composticola*) became dominant and finally, at the most mature stages, the relative importance of the fungal-feeding general opportunists other than Aphelenchoididae, such as Anguinidae (mainly *Ditylenchus filimus*), Neotylenchidae and Tylenchidae increased. Hence, the nematode community actually undergoes a succession of r-strategists, from enrichment opportunists (cp-1) to general opportunists (cp-2), and based on feeding type, from mainly bacterial-feeders via dominance of bacterial-feeding/predators to increased importance of fungal feeders (Figure 8.1). However, regarding the distinct feeding type-related patterns observed here, it is well known that feeding type allocations based on stoma morphology (Yeates et al. 1993) are not always univocal and some species do not restrict to one food source (1.4.4). For instance, bacterial-feeding nematodes might also feed on amoebae or protozoa (Moens et al. 2004) or most diplogasterids are able to feed on other sources next to bacteria (Fürst von Lieven and Sudhaus 2000). Nevertheless, whatever feeding type would be appointed for a given taxon, the same sequence and proportions of specific buccal cavity morphologies recurrently appeared in different compost processes. Determining the exact functional role in the process is important from an ecological point of view, but does not have an essential influence on the observed patterns (Figure 8.1) and the associated possibilities to use nematodes to infer the status of compost.

The replacement of the enrichment opportunists by general opportunists and the increase of fungal-feeding nematodes have also been observed by Ferris and Matute (2003), Wang et al. (2004) and Georgieva et al. (2005) during the decomposition of several plant residues in soil. Nematode succession in compost differs from decomposition processes in soil (Georgieva et al. 2005) mainly in the absence of K-strategists and in the postponed dominance of the Neodiplogasteridae (including *Mononchoides*) in compost compared to soil. This retardation could be explained by the high temperatures during the thermophilic and early cooling phase. The increase in fungivorous nematodes coincides with decreasing C/N ratios during the composting process. It is well known that fungal energy channels predominate when the organic input is characterized by a high C/N ratio, and conversely, bacterial decomposition channels predominate when the organic material has a low C/N ratio (Ruess 2003; Ruess and Ferris 2004). Most likely, when looking at the whole process, during the thermophilic phase the extreme environmental circumstances (i.e. temperature) are the limiting factors for nematode succession, whereas during the maturation phase food resources may be the most selective force driving successional events.

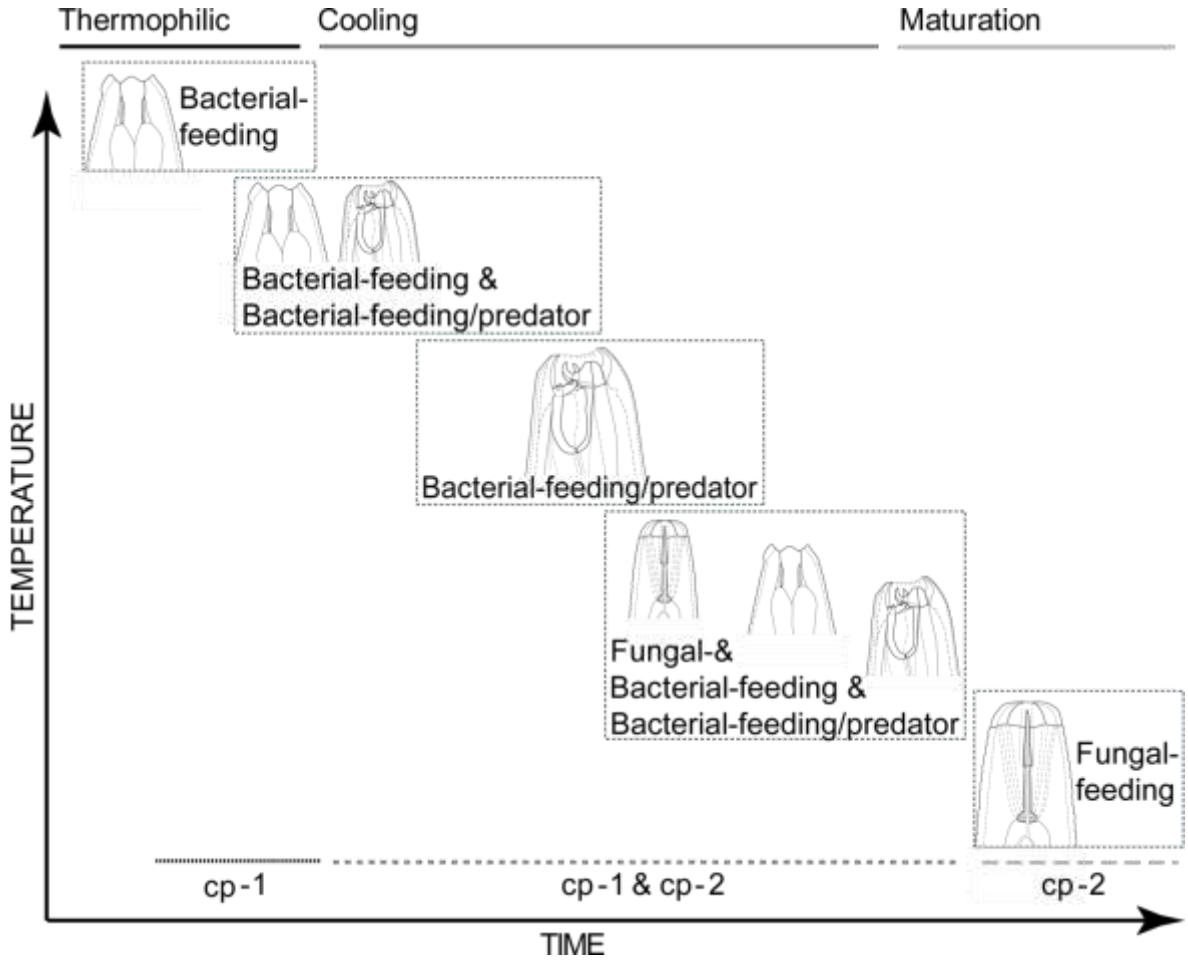


Figure 8.1:
General succession of nematodes during composting. Nematodes with cp-1 and cp-2 are enrichment opportunists and general opportunists, respectively. Nematode feeding types are schematically presented by nematode heads of *Panagrolaimus* sp. (bacterial-feeding), *Ditylenchus* sp. (fungal-feeding), and *Mononchoides composticola* (bacterial-feeding/predator).

Based on this succession of nematodes, nematode-based indices were calculated and their changes during the composting process monitored. Both the Maturity Index (MI) (Bongers 1990, 1999) and variations of the fungivores/bacterivores ratio (*i.e.* F/B ratio (Ferris et al. 2001) in Chapter 2 and F/F+B (Yeates et al., 1993 in Chapter 4) increased as the compost matured. The increased fungivores/bacterivores ratios indirectly indicate decomposition of slowly degradable organic materials such as lignin and lignocellulose at the end of the process, which is known to be mainly executed by fungi (Eiland et al. 2001; Vargas-García et al. 2009). The relation with processing time was less unequivocal in the processes of Chapter 6, where the fungivores/bacterivores indices increased after the thermophilic phase, then decreased and increased again at the end of the process. This is likely due to the fact that temperature in these processes did not show a simple decrease with time as in the other studies. This, however, allowed us to better distinguish between the effect of times and temperature. The resulting data of the replicated design in Chapter 6 were modeled and revealed significant relations of changes in the nematode community (*i.e.* nematode abundance, absolute amount of fungal-feeding nematodes and F/F+B ratio) with other parameters of the composting process (*i.e.* time of composting and/or temperature). The nematode abundances were significantly related with temperature. The abundance of fungal-feeding nematodes was significantly related with both time of composting and temperature, and the F/F+B ratio was only significantly related with time of composting. Addition of PLFA based data (*i.e.* fungal PLFA or PLFA based f/b ratio) as a variable in the appropriate models did not significantly add to the explanation of the observed data, which may be due to the lack of sufficient suitable fungal FA markers (see 8.1). As shown, the nematode succession in compost follows a repeatable and predictable pattern which is related with changes during the composting process and thus is a promising tool to evaluate compost maturity (see 8.3).

Since composting is a microbially mediated process, providing the proper environmental conditions for microbes to decompose raw organic materials is crucial for success (Cooperband 2000). The starting materials have an effect on the chemical compost properties (Chapter 7; Francou et al. 2005; Larney et al. 2008; Grigatti et al. 2011) and determine to a large degree the microbial community composition during composting (Ryckeboer et al. 2003). The chief succession patterns in the nematode community (see 8.2), on the other hand, appeared to be much less dependent on feedstock materials. Irrespective of the used feedstock materials or feedstock mixtures, largely the same nematode successions were observed during virtually all composting processes (Chapter 2, 4, 5 and 6), and very similar nematode community compositions in mature farm composts were found (Chapter 7). Next to feedstock proportion,

differential access to the composting process (covered and uncovered treatments) and scale of composting (barrels versus windrow composting) also did not importantly influence overall nematode succession during composting (Chapter 5). Moreover, the nematode succession was unaffected by the season of composting since processes in different chapters of this PhD were started at different moments in time, from spring to summer (chapter 6) or from autumn to winter (Chapter 2 and 4). Also on genus and species level remarkably similar taxa were found in the examined composts, despite the geographic disparity, differential access, different feedstock materials and method and timing of composting (green *vs.* farm, spring vs autumn) (e.g., the following literature data support the present study: *Eucephalobus*, *Poikilolaimus*, *Diploscapter*, *Aphelenchoides* and *Ditylenchus* were prominent in Gagarin 2000; *Halicephalobus gingivalis* in Nadler et al. 2003; *Panagrolaimus labiatus* in Andrassy 1984). The present study of the compost nematofauna revealed 21 taxa that were new to the Belgian fauna (Chapter 3-Part 1) and included new and numerically very important species, *i.e. Mononchoides composticola* (Chapter 3-Part 2). The genus *Mononchoides* was found in all examined composts and *Mononchoides composticola* was recorded in all farm composts (11). However, in the current study the nematodes were mostly identified only up to genus level. A more thorough and comprehensive taxonomic study might well have revealed several more species new to science and/or to the Belgian fauna.

In contrast to the overall similarity in succession patterns, the nematode successions during industrial green waste composting (Chapter 6) and a finished green waste compost (Chapter 7) were different. The nematode abundances were conspicuously lower and functional group proportions were different because of the virtual absence of fungal feeders. Nevertheless, the limited number of recorded taxa in the green waste composts were also found in the farm processes, except *Procephalobus* sp. which was unique to the industrial green waste compost. The observed difference is most likely mainly based on the composting method. Green waste composting uses standardized procedures for all processes with predetermined time schedules for management practices and duration of the process and depends, compared to the farm composts, on a different waste stream that contains more fresh materials.

8.3 NEMATODES AS INDICATORS OF COMPOST MATURITY

Based on the universal shifts in the nematode community during composting and especially on the distinct increase of the amount of fungal-feeding nematodes (see 8.2), the nematode based indices F/F+B and MI were proposed as suitable tools to assess compost maturity (Chapter 2, 4 and 6). However, a well-accepted parameter of compost maturity to validate our nematode data is not available.

8.3.1 Chemical versus biological maturity

During the compost curing phase (*i.e.* maturation) the microbial community continued to change long after the compost organic matter and other biochemical properties had stabilized (Danon et al. 2008). Ryckeboer et al. (2003) confirmed an increase of microbial diversity after cooling during maturation together with a decline in biological activity at the end of the process indicating stability. Therefore, when considering compost maturity, it should be noted that chemical maturity does not necessarily coincide with biological maturity. Often certain chemical parameters indicate compost maturity shortly after cooling while the biological community still undergoes significant changes during maturation. For example, this was the case in the replicated processes of Chapter 6 where measurements after 13 weeks of composting of the NO_3/NH_4 (>1, Bernal et al. 1998) ratio and OUR (oxygen uptake ratio, >8, Kalamdhad et al. 2008) already indicated chemically mature composts, whereas the nematode community did undergo some significant changes, including the relative increase of fungal-feeding nematodes, after this period. This chemical stability is also nicely illustrated by other measured process parameters like pH and C/N ratio which prominently increased and decreased, respectively, during the thermophilic and cooling phase and afterwards remained stable or only slightly changed during maturation. Apparently, after a certain chemical equilibrium is reached, the compost biota communities (*i.e.* nematodes and microbes) need time to develop. Hence, a proper identification of true compost maturity requires assessment of one or more biological components. In this respect, it is evident that for the industrial green waste compost addition of a more extensive (low-temperature) maturation period would definitely favor nematode as well as microbial abundance.

The quality of the biological component (i.e. both abundance and diversity), though hitherto hardly investigated, may be an explanatory factor for the conflicting findings on the beneficial effects of composts on soil (see also 8.4). The expected benefits of compost application to soil are absent if they lack the correct inoculum of organisms (Ingham and Slaughter 2001). Nematodes possess an important advantage for assessing this component since they are

established environmental indicators (Bongers and Ferris 1999; Neher 2001; Yeates 2003) of underlying ecosystem processes such as organic enrichment (Ferris and Bongers 2006), and changes in the food web are mirrored in shifts in nematode feeding group and taxonomic composition (Yeates et al. 2009).

8.3.2 Nematode-based criteria for compost maturity

Based on the nematode patterns observed in this study and on some specific properties of nematodes, the nematode community has potential as a valuable indicator of the biological component in compost and thus of (biological) maturity. Based on all results obtained in this study, four nematode community-based criteria for biological maturity are proposed (Table 8.1). Based on these criteria a putative simple nematode-based index is proposed. For each fulfilled criterion one point is given resulting in an index with a scale from 0 to 4. Since the nematode abundances are strongly negatively influenced by temperature peaks (Chapter 6), near-ambient temperatures of compost are considered a logical prerequisite for maturity and a correct interpretation of the index. Although temperature decline during composting correlated well with a number of commonly used maturity parameters (e.g. C/N ratio, dehydrogenase activity, ATP content) (Tiquia et al. 2002), depending only on compost temperature may give misleading information (Wichuk and McCartney 2010). Heap temperatures are in fact influenced by various other factors such as ambient conditions, winds, pile size, extreme temperatures during the thermophilic phase, lack of free air space, oxygen depletion, drying and high moisture contents (Lasaridi et al. 2000, Khan et al. 2009).

The first nematode-based criterion we propose is nematode density. High numbers of nematodes are potentially beneficial for maintenance of a solid, balanced and healthy soil food web (see 8.4). An abundance of over 500 ind./ 100g DW compost is proposed here because in the experimental controlled and well balanced farm processes nematode abundance was never below 500/ 100g DW at near-ambient temperatures. In contrast, the biologically poor green waste composts contained far fewer nematodes, *i.e.* maximum 300/ 100g DW in sieved[10] samples. The other criteria take into account the functional composition of the nematode community. As repeatedly mentioned above, the nematode based F/F+B ratio reflects the characteristic increase of fungal-feeding nematodes during maturation and therefore is a suitable ratio to assess maturity. Although the MI also increased during the process (chapter 2 and 4), this index was not included, firstly, because only two out of the five original cp-groups (*i.e.* cp-1 and cp-2, Bongers 1990, 1999) occurred during the process, and secondly because the

[10] It can be expected that densities in sieved compost samples are higher, i.e. the observed density of 300 nematodes/100 g is even overestimated relative to the non-sieved samples.

increase in cp-2 nematodes at the end of the process is almost exclusively caused by the increase of fungal-feeding nematodes which is already accounted for in the fungivores/bacterivores ratio. Based on the tremendous difference between the farm composts and the industrial green waste and the lowest levels obtained for this ratio (*i.e.* 0.003±0.005 in the green waste compared to between 0.37±0.12 and 0.9±0.06 in all other composts), an F/F+B > 0.02 is put forward as a second criterion that needs to be fulfilled. Next to the absolute number of fungivorous nematodes, the diversity of fungal-feeding taxa also increased during the examined composting processes. Immediately after the heat peak, usually only one fungal-feeding taxon (mostly Aphelenchoididae) was present, while later, during maturation, other fungal-feeding taxa appeared. Hence, mature compost can be characterized by at least one additional fungal-feeding taxon next to the early appearing Aphelenchoididae, such as species from the Tylenchidae, Neotylenchidae and/or Anguinidae. The fourth and last criterion is also related to an increase in diversity and includes the presence of diplogasterids (Diplogasteridae *sensu lato*). The presence of diplogasterids provides an easy indication of more trophic diversity (cfr. morphology of the buccal cavity). Diplogasterids are commonly known as bacterial-feeding nematodes, but often they are also able to feed on other organisms such as nematodes, fungi and algae (especially the Neodiplogasteridae) (e.g. Yeates et al. 1993; Fürst von Lieven and Sudhaus 2000; Chapter 3). Hence, diplogasterids may essentially be considered as omnivores *s.l.* to which ecological theory and modeling often attribute a key role in determining food web stability, principally because it increases connectance (Moens et al. 2004) and/or diversity (Gravel et al. 2012). However, according to Dunne et al. (2002), robustness increases with food-web connectance but appears independent of species richness and omnivory.

These four criteria result in a simple index that may also be used by a non-expert after limited training. However, these criteria are still based on a limited dataset of mainly one method of composting (*i.e.* Controlled Microbial farm Composting) in only one geographical region and the index has not yet been tested. In other words, it has not been demonstrated yet that compost with a high index is effectively more beneficial. Hence, the proposed criteria need to be tested, validated and fine-tuned when more data become available.

Table 8.1:
Overview of the proposed nematological criteria to assess biological compost maturity. When all criteria are fulfilled, the compost receives a maximal score of 4/4.

Criteria	Required level
Nematode abundance	> 500/ 100 g DW compost
F/F+B ratio	> 0.2
Number of fungal-feeding taxa	> 1
Presence of diplogasterids	X

As an example of how the proposed nematode-based compost index could work, we calculated the index for all composts analyzed in this study (Table 8.2). The closer the index approaches 4, the better the biological maturity of the compost should be. Following this index all farm composts, with a precise monitoring of the process and careful selection of the feedstock materials, have high scores (*i.e.* 3 or 4). The industrial green waste compost is indicated as biologically very poor. Unfortunately, intermediate processes, between fine-tuned farm composts and large industrial processes, are missing. These are necessary for the interpretation of more subtle differences between composts.

Table 8.2:
Index score based on the proposed criteria for biological maturity of all examined composts in this study. When values are highlighted in grey the respective criterion was not met.

	abundance	F/F+B	Fungal-feeding taxa	diplogasterids	index score
industrial green waste	177 ± 57	0.003 ± 0.005	1	absence	**0**
green waste *	300 ± 40	0.4 ± 0.11	2	X	**3**
poplar bark 1	933 ± 107	0.9 ± 0.06	1	X	**3**
poplar bark 2	722 ± 158	0.5 ± 0.26	2	X	**4**
oak bark*	1700 ± 300	0.52 ± 0.05	2	X	**4**
poplar bark 3*	3100 ± 520	0.67 ± 0.04	2	X	**4**
Norway spruce*	2000 ± 400	0.56 ± 0.06	2	X	**4**
75% poplar bark	1662 ± 157	0.77 ± 0.17	3	X	**4**
50% poplar bark	596 ± 52	0.71 ± 0.09	4	X	**4**
25% poplar bark	768 ± 113	0.37 ± 0.12	3	X	**4**
Willow wood chips*	4000 ± 720	0.86 ± 0.02	3	X	**4**
poplar wood chips*	4400 ± 669	0.73 ± 0.07	2	absence	**3**
poplar bark 4*	900 ± 190	0.83 ± 0.08	2	X	**4**

* only final stage compost available and samples were sieved prior to nematode extraction

8.4 THE IMPORTANCE OF NEMATOFAUNA IN COMPOST AND ITS POTENTIAL BENEFITS

Several compost nematode taxa have a phoretic association with insects, likely to enhance their dispersal (Chapter 5). Rarer compost taxa even have a more specific relation, such as Neotylenchidae sp. or *Ektaphelenchoides* sp. Members of the family Neotylenchidae have two types of generations, a free-living fungal-feeding generation and a female parasitic generation in insect or mite haemocoel. *Ektaphelenchoides* species were recorded as associated with insects in xylem of several woods. Given the distinct relations of compost nematodes with insects and given the fact that insect phoresy is considered an important step towards parasitism (Dillman et al. 2012), compost is an under-investigated habitat for nematodes with bio-control potential. In addition, the diplogasterid predators present in mature composts have been proposed as suitable predators for bio-control of pathogenic nematodes (Khan and Kim 2007). However, at first sight they did not appear to survive in high numbers when added to the soil (Chapter 7). Next to the potential specific role of certain species as biological agents, the nematode community in compost might contribute to a more general and important goal: *i.e.* a solid, balanced and healthy soil food web. Nematodes occupy a central role in the food web and contribute substantially to nutrient cycling in the soil (Verhoef and Brussaard 1990). The ecosystem services of nematodes are determined by their metabolic and behavioral activities and include relocations of organisms to new resources and feeding on other organisms (plants, fungi, bacteria, micro-arthropods and other nematodes), taking in more N than is needed for their body structure (Ferris 2010). Excess N is mineralized as ammonia, excreted and available for uptake by plants and bacteria (Ferris et al. 1998). Abundance, activity and (functional) diversity of the nematode community are important for the continuity of their ecosystem services (Ferris 2010). Our results indicated at least short term survival of compost nematodes in soil as total nematode numbers and especially the fungal feeding *Ditylenchus* increased. Hence, when compost nematodes would also have an effect on the longer run in the soil, compost can be considered as much more than an “ordinary” fertilizer because if nematodes effectively contribute to a more balanced soil food web, the ongoing release of nutrients needed for plant growth might be influenced in the longer run. Previous studies on the suppressing effect of compost on plant-parasitic nematodes mostly did not include free-living taxa in their faunal analyses and completely overlooked the nematode population present in mature composts. However, the proportion of beneficial to harmful nematodes might be more important for plant growth than only the decrease of the absolute number of plant-parasites (Ferris 2010). Thoden et al. (2011) already ascribed the positive effect of compost application to the proliferation of non-pathogenic, free-living nematodes and their overall positive effects on soil microbial

populations, organic matter decomposition, nutrient availability, plant morphology and ecosystem stability, making the plant less susceptible for PPN. Admittedly, more experiments with application of biologically rich and poor composts in the field are needed to elucidate the precise contribution of the compost inhabiting nematodes to the beneficial effects of compost or whether the particular characteristics of compost itself stimulate soil food web processes.

Besides nematodes, compost comprises several other organisms that could have similar or additional beneficial functions. Certain micro-organisms that can be found in compost are thought to play a role in plant disease control. Fungal genera, *i.e. Trichoderma*, *Penicillium* and *Aspergillus*; bacterial genera, *i.e. Bacillus*, *Pseudomonas* and *Pantoea*, and finally actinomycetes are antagonistic to some disease-causing fungi (Hoitink and Boehm 1999). Some of these microbial genera are also known to be suppressive to plant-parasitic nematodes (e.g. Sharon et al. 2001; Kokalis-Burella et al. 2003; Mekete et al. 2009). Also mites are commonly found in compost (own observations and Odegaard and Tommeras 2000) and when acting as a predator might have a structuring effect on the food web.

However, several of these organisms are difficult to detect or require multiple techniques, prohibiting their routine monitoring. As discussed above these beneficial organisms are likely to be only or mostly present in biologically mature compost.

8.5 FUTURE PROSPECTS: WHERE TO GO FROM HERE?

Although important advances have already been made to link features of the nematode succession during composting to compost maturity criteria, some questions remain unanswered. The universality of nematode criteria for composts with different feedstock's, geographical regions and produced by different methods remains to be further investigated. Therefore future endeavors to validate the proposed nematode-based compost maturity index should include and test composts produced with different compost methods from a local to a global scale. Most importantly, the relation of what is here considered as a compost of high quality (compost with a high index) and their effective beneficial aspects needs to be tested. Thoden et al. (2011) hypothesized that the overall positive effect of compost amendments is, inter alia, due to the proliferation of non-pathogenic, free-living nematodes and their overall positive effects on soil microbial populations, mineralization, nutrient supply and plant vitality. This hypothesis can be tested by executing compost application experiments (both short term and long term) with biologically rich and poor composts. Comparison between the effects of nematode poor and rich compost addition will elucidate the precise contribution of compost inhabiting organisms to the

beneficial effects of compost on soil. The validity of this hypothesis is definitely an important consideration for any future research including composts.

Our results indicated that green waste composting resulted in a hyper-sanitized, biologically poor compost. As a guideline to improve the biological quality of this compost we suggest the addition of a low-temperature maturation phase. However, it remains to be investigated whether it is possible for beneficial organisms to colonize these composts after such prolonged periods of high temperatures. In this context we need to know the possibility and the consequences of the colonization of a pathogen. This is certainly not inconceivable in a sterilized and thus nearly antagonist- free compost.

Above the classical use of compost, the investigation of the possibility of enriching compost with specific bio-control agents for specific field problems could be the next important step in compost research. As effective biological regulation requires co-location of predator and prey organisms, or overlaps in their ranges (Ferris 2012), some practical problems frequently preventing successful or sustained biological control might actually be overcome with compost as a carrier. The bio-control agents are readily incorporated into the soil close to their field of action with plenty of resources available for their survival. Experiments on the survival, persistence and reproduction of bio-control organisms are required to further explore this very appealing way of applying bio-control agents. In addition, the effective value (both ecologically as economically) of the compost will enhance tremendously and compost can be more or less designed to tackle specific problems (i.e. pathogens) in the field.

8.6 CONCLUSION

During this project, the nematode community during composting was thoroughly analyzed for the first time. This revealed a repeatable and predictable pattern of nematode succession in all examined processes, except for the industrial green waste compost. This pattern was independent of scale, season of composting, feedstock proportions and also differential access did not importantly influence the nematode succession. In contrast to the microbial community pattern (based on PLFA data), the observed nematode succession was clearly related to changes during the composting process (i.e. composting phases, temperature and duration of composting) and is thus a promising tool to evaluate compost maturity. Four criteria to assess biological compost based on characteristics of the nematode community were proposed maturity: nematode abundances >500/ 100 g DW compost, a F/F+B ratio > 0.2, the presence of more than one fungal-feeding taxon and the presence of diplogasterids. These criteria need to be validated when more data become available. Important steps were taken to understand how

nematodes colonize a compost process, i.e. by a combination of survival strategies and phoretic transport by insects. It has also been demonstrated that the short term effects of compost application on the soil nematode community might be purely caused by an additional effect of compost nematodes.

8.7 REFERENCES

Andrassy, I., 1984. Klasse Nematoda: (Ordnungen: Monhysterida, Desmoscoleaida, Aeraeolaimida, Chromodorida, Rhabditida). Stuttgart: Fischer, p. 228.

Bernal, M.P., Paredes, C., Sanchez-Monedero, M.A., Cegarra, J., 1998. Maturity and stability parameters of composts prepared with a wide range of organic wastes. Bioresource Technol. 63, 91-99.

Bongers, T., 1990. The Maturity Index - An ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Bongers, T., 1999. The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. Plant Soil 212, 13-22.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecol. Evol. 14, 224-228.

Danon, M., Franke-Whittle, I.H., Insam, H., Chen, Y., Hadar, Y., 2008. Molecular analysis of bacterial community succession during prolonged compost curing. Fems Microbiol. Ecol. 65, 133-144.

Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P., Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. PLoS Pathog. 8, 1-4.

Dunne, J.A., Williams, R.J., Martinez, N.D., 2002. Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecol. Lett. 5, 558-567.

Eiland, F., Klamer, M., Lind, A.M., Leth, M., Baath, E., 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microb. Ecol. 41, 272-280.

Ferris, H., Venette, R.C., van der Meulen, H.R., Lau, S.S., 1998. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant Soil 203, 159-171.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18, 13-29.

Ferris, H., Matute, M.M., 2003. Structural and functional succession in the nematode fauna of a soil food web. Appl. Soil Ecol. 23, 93-110.

Ferris, H., 2010. Contribution of nematodes to the structure and function of the soil food web. J. Nematol. 42, 63-67.

Frostegård, A., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biol. Biochem. 43, 1621-1625.

Georgieva, S., Christensen, S., Petersen, H., Gjelstrup, P., Thorup-Kristensen, K., 2005. Early decomposer assemblages of soil organisms in litterbags with vetch and rye roots. Soil Biol. Biochem. 37, 1145-1155.

Gravel, D., Canard, E., Guichard, F., Mouquet, N., 2011. Persistence increases with diversity and connectance in trophic metacommunities. Plos One 6.

Joergensen, R. G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977-2991.

Kaiser, C., Frank, A., Wild, B., Koranda, M., Richter, A., 2010. Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2 omega 6,9 and 18:1 omega 9. Soil Biol. Biochem. 42, 1650-1652.

Kalamdhad, A.S., Pasha, M., Kazmi, A.A., 2008. Stability evaluation of compost by respiration techniques in a rotary drum composter. Resour. Conserv. Recy. 52, 829-834.

Khan, M.A.I., Ueno, K., Horimoto, S., Komai, F., Tanaka, K., Ono, Y., 2009. Physicochemical, including spectroscopic, and biological analyses during composting of green tea waste and rice bran. Biol. Fert. Soils 45, 305-313.

Khan, Z., Kim, Y.H., 2007. A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Appl. Soil Ecol. 35, 370-379.

Kokalis-Burelle, N., Vavrina, C.S., Reddy, V.S., Kloepper, J.W., 2003. Amendment of muskmelon and watermelon transplant media with plant growth-promoting rhizobacteria: Effects on seedling quality, disease, and nematode resistance. Horttechnology 13, 476-482.

Lasaridi, K.E., Stentiford, E.I., Evans, T., 2000. Windrow composting of wastewater biosolids: process performance and product stability assessment. Water Sci. Technol. 42, 217-226.

Mekete, T., Hallmann, J., Kiewnick, S., Sikora, R., 2009. Endophytic bacteria from Ethiopian coffee plants and their potential to antagonise *Meloidogyne incognita*. Nematology 11, 117-127. Ref ID: 511

Moens, T., Yeates, G.W., De Ley, P., 2004. Use of carbon and energy sources by nematodes. In: Cook, R.C., Hunt, D.J. (Eds.), Proceeding of the Fourth International Congress of Nematology, 2002, Spain, Tenerife, 2, 529-545.

Nadler, S.A., Carreno, R.A., Adams, B.J., Kinde, H., Baldwin, J.G., Mundo-Ocampo, M., 2003. Molecular phylogenetics and diagnosis of soil and clinical isolates of Halicephalobus gingivalis (Nematoda: Cephalobina : Panagrolaimoidea), an opportunistic pathogen of horses. Int. J. Parasitol. 33, 1115-1125.

Neher, D.A., 2001. Role of Nematodes in Soil Health and their Use as Indicators. Journal of Nematology 33, 161-168.

Novinscak, A., DeCoste, N.J., Surette, C., Filion, M., 2009. Characterization of bacterial and fungal communities in composted biosolids over a 2 year period using denaturing gradient gel electrophoresis. Can. J. Microbiol. 55, 375-387.

Odegaard, F., Tommeras, B.A., 2000. Compost heaps- refuges and stepping stones for alien arthropod species in northern Europe. Divers. Distrib. 6, 45-59.

Roh, S.W., Abell, G.C., Kim, K.H., Nam, Y.D., Bae, J.W., 2010. Comparing microarrays and next-generation sequencing technologies for microbial ecology research. Trends Biotechnol. 28, 291-299.

Ruess, L., 2003. Nematode soil faunal analysis of decomposition pathways in different ecosystems. Nematology 5, 179-181.

Ruess, L., Ferris, H., 2004. Decomposition pathways and successional changes. Proceeding of the Fourth International Congress of Nematology 2, 547-556.

Ruess, L., Chamberlain, P.M., 2010. The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol. Biochem. 42, 1898-1910.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., Swings, J., 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Ann. Microbiol. 53, 349-410.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O., Spiegel, Y., 2001. Biological control of the root-knot nematode Meloidogyne javanica by Trichoderma harzianum. Phytopathology 91, 687-693.

Shokralla, S.H.A.D., Spall, J. L., Gibson, J.F., Hajibabaei, M.E.H.R., 2012. Next-generation sequencing technologies for environmental DNA research. Mol. Ecol. 21, 1794-1805.

Steger, K., Jarvis, A., Vasara, T., Romantschuk, M., Sundh, I., 2007. Effects of differing temperature management on development of Actinobacteria populations during composting. Res. Microbiol. 158, 617-624.

Steger, K., Sjogren, A.M., Jarvis, A., Jansson, J.K., Sundh, I., 2007. Development of compost maturity and Actinobacteria populations during full-scale composting of organic household waste. J. Appl. Microbiol. 103, 487-498.

Tabatabaei, M., Zakaria, M.R., Rahim, R.A., Wright, A.D., Shirai, Y., Abdullah, N., Sakai, K., Ikeno, S., Mori, M., Kazunori, N., Sulaiman, A., Hassan, M.A., 2009. PCR-based DGGE and FISH analysis of methanogens in an anaerobic closed digester tank for treating palm oil mill effluent. Electron. J. Biotechn. 12.

Thoden, T.C., Korthals, G.W., Termorshuizen, A.J., 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? Nematology 13, 133-153.

Vargas-Garciá, M.C., López, M.J., Suárez-Estrella, F., Moreno, J., 2012. Lignocellulolytic microogranisms from a composting habitat: current and future prospects. In Composting: processing, materials and approaches, eds. J. C. Pereira & J. L. Bolin, pp. 269-294. Nova Science Publishers, Inc., New York.

Verhoef, H.A., Brussaard, L., 1990. Decomposition and Nitrogen Mineralization in Natural and Agroecosystems - the Contribution of Soil Animals. Biogeochemistry 11, 175-211.

von Lieven, A.F., Sudhaus, W., 2000. Comparative and functional morphology of the buccal cavity of Diplogastrina (Nematoda) and a first outline of the phylogeny of this taxon. J. Zool. Syst. Evol. Res. 38, 37-63.

Wang, K.-H., McSorley, R., Marshall, A.J., Gallaher, R.N., 2004. Nematode community changes associated with decomposition of *Crotalaria juncea* amendment in litterbags. Appl. Soil Ecol. 27, 31-45.

Yeates, G.W., Bongers, T., Degoede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding-Habits in Soil Nematode Families and Genera - An Outline for Soil Ecologists. J. Nematol. 25, 315-331.

Yeates, G.W., 2003. Nematodes as soil indicators: functional and biodiversity aspects. Biol. Fert. Soils 37, 199-210.

Yeates, G.W., Ferris, H., Moens, T., Van der Putten, W.H., 2009. The role of nematodes in ecosystems. In Nematodes as environmental indicators, eds. M. J. Wilson & T. Kakouli-Duarte, pp. 1-44. CAB International, Oxfordshire.

Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fert. Soils 29, 111-129.

9. Summary / Samenvatting

9.1 SUMMARY

Composting is the semi-artificial, aerobic, heat producing and controlled process in which microorganisms convert a mixed organic substrate into carbon dioxide (CO_2), water, inorganic nutrients and stabilized organic matter. One of the key issues in compost research is to determine when the compost has reached a mature stage. Hitherto numerous tests, based on both physical and chemical parameters, have been proposed to assess compost maturity but unfortunately, none of these have proven rigorous, reliable or consistent enough to be used in standard protocols. Although the biology of compost (i.e. micro-and mesofauna) is considered crucial for its beneficial effects of compost on soil, the compost fauna remains largely unknown. Only the dynamics of the bacterial community has been thoroughly investigated in relation to composting processes. In this study nematodes were used as gateway to examine the composting ecosystem, making use of their excellent qualities as environmental bio-indicators. Our main aims were to (1) acquire fundamental knowledge on the nematode diversity in compost, (2) obtain insight into the succession of the nematode community during composting and link this to relevant biotic and abiotic factors, (3) assess the survival and colonization capacity of compost nematodes and (4) test the effect of compost with its associated nematodes on the existent soil nematode community and the soil chemical properties.

In total 9 complete composting processes (*i.e.* 5 farm composting processes, 1 industrial green waste process and 3 small-scale processes in compost barrels) and 7 finished composts were examined. Nematodes were consistently found in all investigated composts, and they were effectively omnipresent during virtually all stages of the composting process. Remarkably similar major shifts in nematode assemblage composition occurred in all studied composting processes except the industrial green waste compost. At the beginning of the process (thermophilic phase >45°C), immediately after the heat peak, the nematode population primarily consisted of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae and Diplogasteridae), supplemented with fewer bacterial-feeding general opportunists (cp-2) (Cephalobidae) and/or with the fungal-feeding general opportunists (Aphelenchoididae). Thereafter, during the cooling (<45°C) and maturation stages (near ambient temperatures), first the bacterial-feeding/predator enrichment opportunistic nematodes (mainly *Mononchoides composticola*) become dominant and finally, at the most mature stages, the relative importance of the fungal-feeding general opportunists other than Aphelenchoididae, such as Anguinidae (mainly *Ditylenchus filimus*),

Neotylenchidae and Tylenchidae increased. Hence, the nematode community actually undergoes a succession of r-strategists, from enrichment opportunists (cp-1) to general opportunists (cp-2) and based on feeding type, from mainly bacterial-feeders via dominance of bacterial-feeding/ predators to increased importance of fungal-feeders.

Next to feedstock proportion, also scale of composting (barrels versus windrow composting) did not importantly influence overall nematode succession during composting. Moreover, the nematode succession was unaffected by the season of composting since processes in different chapters of this PhD were started at different moments in time, from spring to summer or from autumn to winter. Also on genus and species level remarkably similar taxa were found in the examined composts, despite the geographic disparity, differential access, different feedstock materials and method and timing of composting (green *vs.* farm, spring vs autumn). The present study of the compost nematofauna during composting revealed 21 taxa that were recorded for the first time in Belgium and a new, numerically very important species, *i.e. Mononchoides composticola* was described. The genus *Mononchoides* was found in all examined composts and *Mononchoides composticola* was recorded in all farm composts.

Based on the remarkable repeatable succession of nematodes, nematode-based indices were calculated. Both the Maturity Index (MI) the fungivorous/bacterivorous ratio (F/F+B) increased as the compost matured. The relation of these indices with progressing time was less unequivocal for three simultaneously running but independent processes, likely due to the fact that temperature in these processes did not show a simple decrease with time as in the other studies. This, however, allowed us to better distinguish between the effect of times and temperature in a generalized linear mixed model. The nematode abundances were significantly related with temperature; the amount of fungal-feeding nematodes was significantly related with both time of composting and temperature, and the F/F+B ratio was only significantly related with time of composting. As demonstrated by our data, the nematode succession in monitored compost processes follows a repeatable and predictable pattern and especially the nematode based F/F+B holds promise as a reliable indicator of compost maturity since it was not significantly affected by temperature fluctuations but mainly related with time of composting.

Compared to the nematode community succession, the changes in the microbial community as based on our PLFA analyses were mostly concentrated in the first month of composting and were less pronounced and not univocal among examined processes. No distinct changes in the major microbial functional groups (gram-positive, gram-negative, actinomycetes and fungi) related with compost maturity were found. Addition of PLFA-based data (*i.e.* fungal-PLFA or PLFA based f/b ratio) as a variable in the models that describe the changes in the nematode community did not

significantly add to the explanation of the observed data, which may be due to the lack of sufficient suitable fungal PLFA markers.

Due to the heat peak early in the compost process nematodes disappeared completely or to a large extend, but several taxa appear immediately when the temperatures drop. These comprise both taxa present before the heat peak and new taxa. Our results showed that composting processes inaccessible to insects and/or not in contact with soil did not significantly influence the principal nematode succession patterns during composting. However, for some specific taxa differences between treatments were found (i.e. for *Achrostichus* sp., Neodiplogasteridae sp., *Nygolaimoides* sp. and Rhabditidae sp.1) illustrating the importance of insects for the dispersal of nematodes to compost. Experiments in the lab with the blue bottle fly as a possible carrier demonstrated actual transport of nematodes isolated from compost by the fly (i.e. *Halicephalobus* cfr. *gingivalis*, *Diploscapter coronatus*, *Diplogasteritus* sp., *Acrostichus* sp., *Mesorhabditis* sp.). The importance of survival during the compost process was demonstrated for *Aphelenchoides* sp., *Panagrolaimus* sp. and rhabditid juveniles and dauer stages, as they survived an experimentally induced temperature peak (up to 60°C) while Tylenchidae did not. Hence, our results indicate that the rapidly changing nematode community in a composting process is the result of both differential survival and colonization capacities.

Previous studies on the effect of compost on the existing nematode communities in soil never took into account the nematodes that are already present in finished composts. Therefore 11 composts (10 farm composts and one green waste compost), with known nematode populations, were further tested in an incubation experiment to investigate their short-term effect on the existent soil nematode community and on the soil chemical properties. After 12 weeks of incubation all compost amendments resulted in a significant pH increase in the soil (one-way ANOVA, $p<0.01$) and also the nematode numbers in the soil increased, except in soil amended with the green waste compost. In particular, the abundance of the fungal-feeding *Ditylenchus* increased significantly (one-way ANOVA, $p<0.03$) in all soils amended with farm compost. This increase may well be purely caused by the addition of nematodes present in the compost and thus results on the effects of compost should be interpreted with caution.

9.2 SAMENVATTING

Compostering verwijst naar het semi-artificiële, aerobe, warmte producerende en gecontroleerde proces waarbij micro-organismen een gemengd organisch substraat omzetten in koolstofdioxide (CO_2), water, anorganische voedingsstoffen en gestabiliseerde organische stof (humus). Een van de belangrijkste vragen in compostgerelateerd onderzoek is: wanneer bevindt compost zich in een rijp stadium? Tot hiertoe werden tal van tests voorgesteld om de rijpheid van compost te bepalen, en deze zijn veelal gebaseerd op fysische en chemische parameters. Helaas is geen enkele van deze tests nauwkeurig, betrouwbaar en consistent genoeg om gebruikt te worden als enige maatstaf voor maturiteit van compost in standaardprocedures. Hoewel de biologie van compost (zowel micro- als mesofauna) beschouwd wordt als cruciaal voor de gunstige effecten van compost op de bodem, blijft de aanwezige fauna in compost grotendeels onbekend. Enkel de dynamiek van de bacteriële gemeenschap in relatie tot compostprocessen werd al grondig bestudeerd. In deze studie werden nematoden gebruikt als middel om inzicht te verkrijgen in het composteringsecosysteem, steunend op hun uitstekende eigenschappen als bio-indicatoren. Onze belangrijkste doelstellingen waren om (1) fundamentele kennis te verwerven over de diversiteit van nematoden in compost, (2) inzicht te krijgen in de successie van de nematodengemeenschap tijdens de compostering en dit te koppelen aan relevante biotische en abiotische factoren, (3) de overleving en kolonisatie-capaciteit van compostnematoden te bepalen en (4) te testen wat het effect is van compost, die reeds nematoden bevat, op de bestaande nematodengemeenschap in de bodem en op de chemische bodemeigenschappen.

In totaal werden negen volledige composteringsprocessen (i.e. vijf boerderij-composteringsprocessen, één industrieel-groenafvalproces en drie kleinschalige processen in compostvaten) en zeven afgewerkte composten onderzocht. Nematoden werden consequent aangetroffen in alle onderzochte composten en waren effectief alomtegenwoordig in vrijwel alle fasen van het composteringsproces. Behalve in de industriële groencompostering deden er zich opvallend vergelijkbare verschuivingen in de samenstelling van de nematodengemeenschap voor in alle onderzochte composteringsprocessen. Aan het begin van het proces (thermofiele fase> 45 °C), onmiddellijk na de temperatuurpiek, bestond de nematodenpopulatie voornamelijk uit bacterie-etende aanrijkingsopportunisten (cp-1) (Rhabditidae, Panagrolaimidae en Diplogasteridae), aangevuld met minder bacterie-etende (Cephalobidae) en/of schimmel-etende (Aphelenchoididae) algemene opportunisten (cp-2). Daarna tijdens de afkoelings- (<45 °C) en rijpingsfases (bij omgevingstemperatuur), zijn eerst de bacterie-etende/predator

aanrijkingsopportunisten (voornamelijk *Mononchoides composticola*) dominant. Tenslotte neemt in de meest rijpe stadia het relatieve belang van de schimmeletende algemene opportunisten toe, naast Aphelenchoididae, zoals Anguinidae (voornamelijk *Ditylenchus filimus*), Neotylenchidae en Tylenchidae. De nematodengemeenschap ondergaat dus eigenlijk een transformatie van r-strategen of aanrijkingsopportunisten (cp-1) naar algemene opportunisten (cp-2) en gebaseerd op voedingstype, van voornamelijk bacterie-etende nematoden via dominantie van bacterie-etende/predators naar een gemeenschap met een belangrijk aandeel schimmeletende nematoden.

Zowel de gebruikte grondstoffen als de omvang van de compostering (vaten versus compostering op grote schaal) hadden geen belangrijke invloed op de algemene nematodensuccessie. De nematodensuccessie werd bovendien ook niet beïnvloed door het seizoen van compostering omdat de processen in de verschillende hoofdstukken van dit doctoraat gestart werden op verschillende momenten in de tijd, van de lente tot de zomer of van de herfst tot de winter. Ondanks de geografische verschillen, de verschillen in toegang, de verschillende grondstoffen, de verschillen in werkwijze (groen vs. boerderij) en timing van de compostering (lente vs. herfst), werden ook op genus- en soortniveau opvallend gelijke taxa gevonden in de onderzochte composten. De huidige studie over de nematofauna tijdens compostering onthulde 21 taxa die voor de eerste keer werden waargenomen in België waarvan één nieuwe, numeriek zeer belangrijke soort, namelijk *Mononchoides composticola*, die werd beschreven. Het geslacht *Mononchoides* werd gevonden in alle onderzochte composten en *Mononchoides composticola* werd aangetroffen in alle boerderij-composten.

Steunend op de steeds terugkerende opeenvolging van nematoden-assemblages tijdens het composteringsproces werden nematodengebaseerde indices berekend. Zowel de Maturity Index (MI) als de fungivorous/bacterivorous ratio (F/F + B) stijgen tijdens het composteringsproces als de compost rijpt. De relatie van deze indices met de composteringstijd was minder eenduidig bij drie gelijklopende maar onafhankelijke processen. Waarschijnlijk is dit te wijten aan het feit dat, in tegenstelling tot de andere processen, de temperatuur niet eenvoudig afnam met de tijd. Dit liet ons echter toe een beter onderscheid te maken tussen het effect van tijd en temperatuur in een gegeneraliseerd gemengd lineair model. De nematodenaantallen waren significant gerelateerd met de temperatuur, de hoeveelheid schimmeletende nematoden was significant gecorreleerd met zowel composteringstijd als temperatuur, en de F/F+B ratio was enkel significant gecorreleerd met de composteringstijd. Zoals blijkt uit onze gegevens, volgt de nematodengemeenschap tijdens compostering een herhaalbaar en voorspelbaar patroon en vooral de nematodengebaseerde F/F+B ratio is veelbelovend als betrouwbare indicator van

compostmaturiteit, omdat deze niet significant beïnvloed werd door temperatuurschommelingen, maar voornamelijk door composteringstijd.
In vergelijking met de successie van de nematoden, concentreerde de veranderingen in de microbiële gemeenschap (op basis van PLFA analyses) zich vooral in de eerste maand van het composteringsproces en waren ze bovendien minder uitgesproken en niet eenduidig binnen de onderzochte processen. In de belangrijkste microbiële functionele groepen (gram-positieve bacteriën, gram-negatieve bacteriën, actinomyceten en schimmels) werden geen duidelijke rijpheids-gerelateerde veranderingen gevonden. Toevoeging van PLFA-gebaseerde data (d.w.z. schimmel-PLFA of PLFA gebaseerd f/b-verhouding) als variabele in de modellen die de veranderingen van de nematodengemeenschap beschrijven, droegen niet significant bij tot de verklaring van de waargenomen patronen. Dit kan onder andere te wijten zijn aan het ontbreken van voldoende geschikte PLFA merkers voor schimmels.

Als gevolg van de temperatuurpiek vroeg in het composteringsproces verdwenen de nematoden geheel of voor een groot deel, maar zodra de temperatuur zakt, verschijnen er opnieuw verschillende taxa. Hiertoe behoren zowel taxa die al aanwezig waren voor de temperatuurpiek als nieuwe taxa. Onze resultaten demonstreren dat de algemene nematodensuccessie tijdens het composteringsproces niet significant beïnvloed wordt door composteringsprocessen die ontoegankelijk zijn voor insecten en/of niet in contact staan met de bodem. Voor een aantal specifieke taxa werden er wel verschillen gevonden tussen de behandelingen (i.e. voor *Achrostichus* sp., Neodiplogasteridae sp., *Nygolaimoides* sp. en Rhabditidae sp.1). Deze verschillen illustreren het belang van insecten voor de verspreiding van nematoden in en naar compost. Experimenten in het laboratorium met de blauwe bromvlieg als een mogelijke drager toonde daadwerkelijk transport door vliegen aan van verschillende nematoden (*Halicephalobus* cfr. *gingivalis*, *Diploscapter coronatus*, *Diplogasteritus* sp., *Acrostichus* sp., *Mesorhabditis* sp.), geïsoleerd uit compost. Het belang van overleving tijdens het composteringsproces werd aangetoond voor *Aphelenchoides* sp., *Panagrolaimus* sp. en dauers en juvenielen van rhabditiden omdat ze een experimenteel geïnduceerde temperatuurpiek (tot 60 °C) overleefden, terwijl tylenchiden dit niet konden. Onze resultaten wijzen er dus op dat de snel veranderende nematodengemeenschap in een composteringsproces het gevolg is van zowel differentiële overleving alsook van de kolonisatiecapaciteiten van de aanwezige nematoden.

Eerdere studies naar het effect van compost op de nematodengemeenschap in de bodem hielden nooit rekening met de nematoden die reeds aanwezig waren in de toegevoegde compost. Daarom werden elf composten (tien boerderijcomposten en één groenafvalcompost),

met gekende nematodenpopulaties, verder getest in een incubatie-experiment om hun kortetermijneffect op de bestaande nematodengemeenschap in de bodem en zijn chemische eigenschappen te onderzoeken. Na twaalf weken incubatie resulteerde alle toevoegingen van compost, behalve voor de behandeling met groen compost, in een significante toename van de pH(one-way ANOVA, $p < 0,01$) en een algemene stijging van het aantal nematoden in de bodem. Vooral de hoeveelheden van de schimmel-etende *Ditylenchus* namen aanzienlijk toe in alle bodems verrijkt met boerderijcompost (one-way ANOVA, $p < 0,03$). Deze toename kan echter ook uitsluitend veroorzaakt zijn door de nematoden die werden toegevoegd met compost en de resultaten over de effecten van compost moeten dus met de nodige voorzichtigheid geïnterpreteerd worden.

List of publications

PUBLICATIONS IN SCI-INDEXED JOURNALS

- Fonderie P., **Steel. H.**, Moens T., Bert W. (2012). Experimental induction of intraspecific morphometric variability in a single population of *Halicephalobus* cf. *gingivalis* may surpass total interspecific variability. Nematology in press.

- **Steel H.**, Vandecasteele B., Willekens K., Sabbe K., Moens T., Bert W. (2012). Nematode communities and macronutrients in composts and compost-amended soils as affected by feedstock composition. Applied Soil Ecology 61: 100-112.

- **Steel H.**, Moens T., Scholaert A., Boshoff M., Houthoofd W., Bert W. (2011). *Mononchoides composticola* n. sp. (Nematoda: Diplogastridae) associated with composting processes: morphological, molecular and autecological characterization. Nematology 12(3): 347-363.

- **Steel H.**, de la Peña E., FonderieP. , Willekens K., Borgonie G., Bert W. (2010). Nematode succession during composting and the potential of the nematode community as an indicator of compost maturity. Pedobiologia 23(3): 181-190.

- Fonderie P., Willems M., Bert W., Houthoofd W., **Steel H.**, Claeys M., Borgonie G. (2009). Intestine ultrastructure of the facultative parasite *Halicephalobus gingivalis* (Nematode: Panagrolaimidae). Nematology 11(6): 859-868.

OTHER PUBLICATIONS

- **Steel H.**, Bert W. (2011). Biodiversity of compost mesofauna and its potential as an indicator of the composting process status. Accepted for publication in: Special Issue on Compost III in Dynamic Soil, Dynamic Plant 5 (Special Issue 2): 45-50.

ACTIVE CONTIRBUTIONS TO INTERNATIONAL CONFERENCES

- 31st International Symposium of the European Society of Nematologists, Adana, Turkey (2012). Oral presentation:
  "Nematodes in a rapidly changing environment: colonization, microbial links and functional succession in compost." Steel H., Moens T., Buchan D., Fonderie P., De Neve S., and Bert W.

- 2nd International symposium on nematodes as environmental bio-indicators, Ghent, Belgium (2012). Oral presentation:
  "Nematode community during composting and its potential as indicator of compost maturity." Steel H., Moens T., and Bert W.

- 17th Benelux Congress of Zoology, Ghent, Belgium (2010). Oral presentation: "Nematodes as potential bio-indicator of the composting process." Steel H., Moens T., Buchan D., Houthoofd W., De Neve S., and Bert W.

- 30th International Symposium of the European Society of Nematologists, Vienna, Austria (2010). Oral presentation:

"Nematode community during composting and its potential as indicator of compost maturity." Steel H., Moens T., Buchan D., Houthoofd W., De Neve S., and Bert W.

- 5th IOBC meeting on Multitrophic Interactions in Soil, Uppsala, Sweden (2009). Oral presentation:
  "Nematode community as potential indicator of compost maturity and quality." Steel H., de la Peña E., Scholaert A., Boshoff M., Borgonie G., and Bert W.

- 61th International Symposium on Crop Protection, Ghent, Belgium (2009). Poster: "Nematode community as potential indicator of compost maturity and quality." Steel H., de la Peña E., Fonderie P., Willekens K., Borgonie G., Bert W.

- 5th International Congress of Nematology, Brisbane, Australia (2008). Poster: "Nematode succession during Controlled Microbial Composting." Steel H., Bert W., de la Peña E., Fonderie P., Willekens K., and Borgonie G.