



## Original Articles

# Factors influencing the nematode community during composting and nematode-based criteria for compost maturity



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## ABSTRACT

Pilot studies indicate that shifts in the nematode species composition, life strategies and feeding behavior during composting appear to be fairly consistent and, therefore, promising as a potential tool to assess compost maturity. However, this has been only based on a limited number of, mainly, non-replicated observations. In this study, we tested whether the nematode community succession patterns are recurrent for parallel processes and assessed the relationship between the changes in the nematode community and potential important variables (i.e., temperature, duration of composting and the microbial community). The nematode and microbial community of three simultaneously running Controlled Farm Composting and a reference Green Waste composting process were analyzed through time. Bacterial-feeding enrichment opportunists were most numerous during and directly after the heat peaks. Subsequently, the bacterial-feeding/predator community dominated and the fungal-feeding nematodes became more dominant during maturation, confirming general community patterns from previous experiments. Nematode abundances significantly fluctuated with temperature and the relative abundance of fungal-feeding nematodes increased as the duration of the curing process increased. The amount of fungal-feeding nematodes was associated significantly with both duration of composting and temperature, and the F/(F + B) ratio was only significantly associated with duration of composting. Based on these results, and additional data from an industrial reference compost process and on available literature, a Nematode-based Index of Compost Maturity (NICM) is proposed, combining four nematode-based criteria (i.e., nematode abundance, F/(F + B) ratio, the presence of more than one fungal-feeding taxon and the presence of diplogasterids). Nevertheless, the NICM should be considered as work in progress which should be tested for a wider range of composts from diverse feedstock mixtures, locations (sites) and composting techniques, to validate the use of the index and allow more reliable interpretation of particular values of this index.

## 1. Introduction

Composting is an aerobic, heat-producing and controlled process in which microorganisms convert a mixed organic substrate into CO<sub>2</sub>, water, inorganic nutrients and stabilized organic matter. The final compost must be of high quality, i.e., stable, mature and free of health and environmental risks (Cesaro et al., 2015; Wichuk and McCartney, 2010), to be considered beneficial for the soil or to be responsible for associated advantages like improved nutrient capacity of the soil (Tognetti et al., 2008) and disease suppressive activity (Mehta et al., 2014; Oka, 2010). The quality of the organic matter and hence the value as fertilizer, the physical characteristics and the biology (i.e.

inhabiting organisms) are responsible for these beneficial effects. Next to sufficiently high temperature and thus, adequate sanitization as a prerequisite, key issues in compost research and crucial for compost quality assessment are the maturity and stability measures used to evaluate the composting process. Maturity is a general term describing the suitability of the compost for a particular end use, while stability can be defined as the extent to which readily biodegradable material has decomposed (Gomez et al., 2006). Compost stability is usually assessed using a measure for the activity of the microbial community (Neher et al., 2017; Wichuk and McCartney, 2010). Nevertheless, maturity is often informally defined as the state in which compost is dominated by humic substances (Dinel et al., 1996) or when the

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temperature reaches a near-ambient level (Cooperland, 2000). For the past decade, researchers have proposed multiple chemical, physical (Sellami et al., 2008; Zmora-Nahum et al., 2005) and biological parameters (Gomez et al., 2006) to assess compost maturity. To the best of our knowledge, the hitherto proposed tests are imprecise, unsuitable for a wide range of input materials, and incapable of quantifying both compost maturity and stability (Wichuk and McCartney, 2010).

The taxa most essential to the composting process are bacteria, algae, fungi, Isopoda, Acari, Nematoda and protozoans (Cooperland, 2000; Young et al., 2005). This wide spectrum of organisms makes up a complex and rapidly changing community. Of all these taxa, only nematodes (Steel et al., 2013a; Steel et al., 2010) and microbial communities (i.e., bacteria, actinobacteria and fungi) (Ryckeboer et al., 2003; Steel et al., 2013a) are ubiquitous in all stages of the composting process, making them the key groups to monitor. A significant advantage of using nematodes to assess compost maturity is their established status as environmental indicators (Bongers and Ferris, 1999; Neher, 2001; Yeates, 2003) of ecosystem processes such as organic enrichment (Ferris and Bongers, 2006); moreover, changes in the food web are mirrored in shifts in nematode feeding group and taxonomic composition (Yeates et al., 2009). According to pilot studies based on large-scale farm composting systems and small-scale processes in compost barrels (Steel et al., 2013a; Steel et al., 2010; Steel et al., 2013b), shifts in nematode species composition, life-history strategies and feeding behavior occur during the process of composting. At the beginning of the process, during the thermophilic phase, the nematode community is primarily comprised 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 (Aphelenchoiidae), and finally, after a transient dominance of bacterial-feeders/predators (Neodiplogasteridae) in the cooling phase, fungal-feeding general opportunists (Anguinidae and Aphelenchoiidae) become more dominant during the maturation phase. This increasing proportion of fungal-feeding nematodes during the composting process has been proposed as a potential indicator of compost maturity (Steel et al., 2013a; Steel et al., 2010). Compared to the nematode community, the shifts in the microbial community structure, as revealed by phospholipid fatty acids (PLFA), were less pronounced and mostly restricted to the first month of composting (Steel et al., 2013a).

Although nematode community succession appears to be consistent and promising as a tool to assess compost maturity, these patterns have hitherto been based on only a limited number of observations. A better insight into the underlying factors that cause these remarkable shifts in composition of nematode communities, such as processing time, compost temperature and/or microbial community structure, requires parallel controlled composting experiments (Steel et al., 2010; Steel et al., 2013a). In this study, the nematode and microbial community of three simultaneously running controlled farm composting processes, with different proportions of feedstock materials, were monitored through time to assess a) whether the nematode community succession patterns were consistent across different composting processes; and b) the relationship of nematode community changes with variables such as temperature, duration of composting, and the microbial community. Our second main goal was to translate the obtained process-based insights together with literature data into criteria for biological compost maturity. A single industrial green waste composting process was also sampled as a reference of industrial scale composting for comparison with the smaller-scale experimental farm composts.

## 2. Materials and methods

### 2.1. Composting sites and sampling

Three composts were produced simultaneously according to the on-farm Controlled Microbial Composting (CMC)-method (Diver, 2004) in

open-air windrows covered with semipermeable fabric when needed on a concrete floor at the experimental farm of the Institute for Agricultural and Fisheries Research (ILVO at Merelbeke, Belgium). These composts will be referred to as Farm 1, 2 and 3. Three compost windrows (each 15 m long, 3 m wide and 1.5 m high with 3 m<sup>3</sup> feedstock materials per meter) were established with different ratios of hay and ground poplar bark, i.e., 25/75%, 50/50% and 75/25% (vol/vol), respectively. The hay was a mixture of grass and clover hay in which the amount of grass hay in the three compost piles was 0%, 23% and 46% (vol/vol), respectively. Other than the feedstock material (not part of current research question), the experimental conditions of the three composts are identical so that the variability associated with the studied patterns can be estimated. Other than the feedstock material, which was not part of current research question, the experimental conditions of the three composts are identical and therefore treated as replicates to study the temporal patterns in nematode community composition. Each composting process was managed individually, based on monitored temperature, moisture content and CO<sub>2</sub> levels. Urea was added at the start in all three composts to decrease the C/N ratio of the feedstock towards 30:1, which is considered an ideal starting ratio for composting (Zorpas et al., 2009), and cane molasses plus spoiled ensilaged maize were mixed in the feedstock as a compost starter. The windrows were turned on days three and ten to avoid excessive CO<sub>2</sub> concentrations. On day 83 of the composting process, the windrows were moved and stored in three piles for further maturation. The water content of the composting piles was controlled by opening or closing the semipermeable fabric covers 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 mixture (day 0) and on days 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 as further detailed below. Positive effects on crop performance (D'Hose et al., 2012) and soil quality (D'Hose et al., 2014; Willekens et al., 2014) were found for farm composts that were produced at the same site as the composts in this study, which is used as a basis to assign them a high quality status.

The reference industrial green waste compost was produced by the Inter-municipal Society of Public Health in Moen, Belgium. The composting process consisted of five phases. During the first phase a mixture of available feedstock materials was made (i.e., 35% grass, 15% mixed green waste, 40% wood chips, 10% roots of trees). These materials ( $\pm 700 \text{ m}^3$ , 450 kg/m<sup>3</sup>) were then placed into a long windrow (50 m long, 8 m wide and 3 m high) and covered with a semipermeable fabric cover for four weeks. Afterwards, the cover was removed, water was added (25,000 L) and the windrow was turned mechanically (phase 2). Then the windrow was covered again, turned after two weeks (phase 3) and subsequently kept uncovered and turned at three-week intervals (phase 4) to mature. Finally, the compost was sieved and the fraction < 15 mm was sold as compost (phase 5). Sampling took place from the freshly mixed materials (day 0 = phase 1) and during every turning event in each phase (on days 33, 39, 61 and 83 in phase 2,3,4 and 5 respectively). Three composite samples were taken per sampling event.

Each composite sample consisted of 20 thoroughly-mixed and randomly-chosen samples (50 mL each in the farm composts and 1 L each in the reference green waste compost), and of this total volume (respectively 1 L and 20 L), a subsample of 400 mL was taken for nematode extraction (Been et al., 2006). Another portion of each composite sample ( $\pm 600 \text{ mL}$ ) was freeze dried (Christ, Gamma 1–20, Osterode am Harz, Germany), ground and stored frozen for carbon (C), nitrogen (N) and Phospholipid Fatty Acids (PLFA) analyses.

### 2.2. Abiotic variables

Temperature and CO<sub>2</sub> content of the farm composts were measured at three random locations in the windrow using specialized equipment (Digital Thermometer GTH 1150 and Brigon Messtechnik D-63110

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