



Resilience of soil microbial and nematode communities after biofumigant treatment with defatted seed meals



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ABSTRACT

The use of alternative biocidal compounds to replace chemical pesticides after the Directive 2009/128/EC has raised renewed interest in the biofumigation technique. In particular, the defatted seed meals (DSM) derived from *Brassicaceae* plant tissues with high glucosinolate content represent an efficient practice to control soil-born plant pathogens and pests that can be applied in synergy to catch crop green manures. For a wider and safer application of this technique, the impacts on non-target soil microorganisms and free-living nematodes have to be investigated in more depth. In this pot-scale experiment a naturally nematode-infected soil was amended with a glucosinolate-containing DSM from *Brassica carinata*, a non-glucosinolate-containing DSM from sunflower and the metam-sodium fumigant. Tomato plants were transplanted and checked for the presence of pests and/or pathogens and plant vigour. The response of soil microbial communities was assessed by PCR-DGGE analysis of bacterial 16S rRNA and fungal 18S rRNA genes, whereas nematode indices were applied to assess their community structure 0, 10, 32 and 62 days after the treatments. Significant shifts were observed among both bacterial and fungal communities, whereas various changes of nematode communities occurred depending on the nematode family. Similar changes initially occurred in both bacterial and fungal community structure in response to DSM and VAP amendments, but after 62 days fungal communities were more strongly shaped by VAP fumigation than bacteria. The non-biofumigant SUN treatment added organic matter into the soil inducing significant changes in microbial communities, but it was not effective against *Meloidogyne incognita* root infestation. Although the free-living nematode structure was negatively influenced by all treatments, *B. carinata* DMS proved the best compromise between efficiency to control *M. incognita* and environmental impact. These results confirmed the interesting potential of biofumigant DSM amendments as alternatives to chemical fumigants for a more environment-friendly control of some soil-borne diseases.

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1. Introduction

In these last few years the European Community has defined new approaches on the use of pesticides, through the Directive

2009/128/EC that introduced restricted limits for the registration of new pesticides, the phase-out of conventional products characterized by a high environmental impact, as for the substances that deplete the ozone layer (UNEP, 2012) or for their biocidal and toxicological properties (Rasmussen and MacLellan, 2001). At the same time, the EC strongly encourages non-chemical alternatives in agriculture through a new revival of virtuous agronomic techniques (such as rotation), more attention to the organic matter content of soils (by the so-called soil conservation techniques), and the use of natural products in plant management and defense. The common aim of all these approaches is to maintain agricultural productivity even with a lower application of chemicals through the

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improvement of soil fertility and plant health. In this framework, twenty years of studies have highlighted the potential benefits derived from the use of *Brassicaceae* plants and materials in the so called “Biofumigation” technique, which provide pest and disease suppression by glucosinolate-containing plants (Kirkegaard et al., 2000; Kirkegaard and Matthiessen, 2004; Lazzeri et al., 2013). In their native form, glucosinolates do not show any biological activity while, in the presence of water and of the endogenous enzyme myrosinase (β thioglucoside Glucohydrolase EC 3.2.1.147), they are quickly hydrolyzed with the production of a series of breakdown products, mainly isothiocyanates (Manici et al., 1997; Lazzeri et al., 2004; Agerbirk and Olsen, 2012). Due to these features, *Brassica carinata* and other *Brassicaceae* have been widely studied, and after a patented procedure (Lazzeri et al., 2010). Isothiocyanates released from defatted seed meals (DSMs) have shown a clear amendment activity for the containment of soil borne fungi (Sanchi et al., 2005; Larkin and Griffin, 2007; Matthiessen and Kirkegaard, 2006), nematodes (Lazzeri et al., 2009; Zasada et al., 2009) and wireworms (Furlan et al., 2010). Nevertheless the suppression of soil-borne pathogens and pests induced by such biofumigants has also been linked to factors other than isothiocyanates. For example, the enhanced competition of soil-borne pathogens with copiotrophic soil microorganisms favoured by the addition of fresh organic matter (Friberg et al., 2009; Larkin and Honeycut, 2006) or enhanced nitrification of ammonia-oxidizing bacteria leading to NO production which is known to contribute to disease suppression through activating certain plant defence responses (Cohen et al., 2005; Cohen and Mazzola, 2006).

So far, most studies concerning biofumigation have been focused on its efficacy against soil-borne pathogens and pests, while the effects on the structure and function of non-target soil microorganism have been poorly investigated. Although it is well known that microorganisms like bacteria and fungi are the main drivers of several soil processes such as nutrient cycling, decomposition of organic residues, formation of humic substances, and pollutant degradation (Nannipieri et al., 2003; Van der Heijden et al., 2008), our understanding of the effects of biofumigation on such organisms is very limited. Some works reported significant alterations of microbial community structure related to glucosinolates (Bressan et al., 2009; Hollister et al., 2012; Reardon et al., 2013) but both bacterial and fungal communities responded differentially to both DSM type/glucosinolate content and to the availability of amended fresh organic matter (Ochiai et al., 2008; Omirou et al., 2011). Moreover, soil free-living nematodes, which also play important roles in soil functioning, such as grazing on bacteria and fungi and thus regulating decomposition and nitrogen mineralization in the soil ecosystem (Yeates and Coleman, 1982; Seastedt et al., 1988; Sohlenius et al., 1988), might also be significantly affected (Bongers, 1990; Ramirez et al., 2009). Reardon et al. (2013) found that *Brassica juncea* did not enhance nematode abundance, demonstrating that nematocidal activity balanced the potential stimulatory effects of the introduced organic matter. Moreover, these authors found various changes in nematode community structure in relation to the seed meal amendment used: *Brassica napus* favoured *Xiphinema* spp. plant parasitic nematode, while *B. juncea* favoured bacterial and fungal feeders.

The aim of this work was (1) to investigate the impact of a natural biofumigant based on *B. carinata* DSM on the structure of both microbial communities and free-living nematodes, (2) to compare these effects with those induced by the addition of organic matter represented by a non-biofumigant DSM (sunflower) and with a standard commercial soil fumigant (VAPAM). In order to achieve such goals, the effects of DSM's incorporation on the soil microbial community and free-living nematodes were investigated by using a soil naturally infested with the root-knot nematode *Meloidogyne incognita* (Kof. & White) Chitw., an important worldwide pest

of several crops responsible for estimated losses of more than 80 billion Euros/year (McGuire, 2003; Djian-Caporalino et al., 2011). Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA and 18S rRNA genes was used to determine both bacterial and fungal community structure, respectively, whereas several nematode indices were applied to better understand their community structure in the agricultural soil system: the nematode maturity index and plant parasite index are useful means to differentiate nematode communities in soils collected from different management systems (Bongers, 1990), while the Basal index, Enrichment index, Structure index, and Channel index add information on functional guilds to develop the food web (Ferris et al., 2001).

2. Materials and methods

2.1. Experimental design

To evaluate the effect of DSM incorporation, a pot trial was conducted in 2013 on tomato plants grown in a soil naturally infested with *M. incognita*, compared to a soil fumigated with metam-sodium and to an untreated control. At the end of the trial, a phytosanitary evaluation of the tomato plants was also carried out.

The experiment was performed in a greenhouse maintained at $22 \pm 2^\circ\text{C}$ and located in Bologna, Italy ($44^\circ30'27''\text{N}$; $11^\circ21'5''\text{E}$, 54 m above sea level). Four pots (height 12 cm and diameter 14 cm) for each treatment were filled with 1 kg of an agricultural soil collected at Altedo (Ferrara). It was a sandy-loamy soil (clay 13%, silt 18%, sand 69%) with an organic matter amount of 0.82% (according to the Walkley–Black method, 1934) and infested with *M. incognita*, about 50 second stage juveniles (J2s) in 100 ml of soil at the beginning of June. A filter paper was inserted at the bottom of each pot, to prevent *M. incognita* juveniles escaping by irrigation water during the trial. The tested pots were prepared in three replicates with the following treatments:

- 1) untreated soil (CTRL);
- 2) soil treated with 2.1 g per pot of *B. carinata* DSM (CAR), a dose equivalent to a full field application of 3 t ha^{-1} . The DSM was uniformly mixed in the soil before watering;
- 3) soil treated with 2.1 g per pot of sunflower DSM (SUN), a dose equivalent to a full field application of 3 t ha^{-1} . The DSM was uniformly mixed in the soil before watering;
- 4) soil treated with 2.31 ml per pot of VAPAM (metam-sodium) liquid solution diluted in 50 ml of water (VAP). The dose was equivalent to a full field application of 1500 kg ha^{-1} .

After the treatments, CTRL, CAR and SUN samples were irrigated with 100 ml of water per pot to reach field capacity, while the VAP samples were irrigated with only 50 ml, taking into account the water (50 ml) used for the metam-sodium dilution. The pots were then irrigated with 30 ml of water by a drip irrigation test every two days throughout the trial.

Soil samples used for molecular analysis were collected at the beginning of the trial before the first watering (T0) and then 10 (T1), 32 (T2), and 62 (T3) days after the beginning of the trial and were stored at -20°C until analysis. After T1 sampling, four young plants of *Solanum lycopersicum* L. cv. UC82 (ISI Sementi, Parma – Italy), a tomato variety susceptible to *M. incognita*, were transplanted with bared roots in each pot. Soil and plant samples used for nematode community analysis and phytosanitary evaluation, respectively, were collected at T2 and T3 and stored at 4°C until analysis. At each sampling time, four plants for each treatment were carefully removed from the pot, washed from soil and evaluated for plant height (cm) and weight on fresh matter of the whole plant (g) as a way of evaluating their vigour in a non-destructive

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