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Release of isothiocyanates does not explain the effects of biofumigation with Indian mustard cultivars on nematode assemblages

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ABSTRACT

While soil biota play an essential role in ecosystem services, the plant-pathogenic fraction may have a large, negative economic impact on food and feed production. For decades, the use of so-called fumigants (=general biocides) has been a common practice for controlling soil pathogens, including plant-parasitic nematodes. Due to their adverse environmental impact, many fumigants have been banned. Biofumigation – a possible alternative – encompasses the incorporation of mulched Brassicaceous debris into topsoil, and its mode of action is based on the conversion of glucosinolates (GSLs) into nematicidal isothiocyanates (ITCs). Contrary to the relatively well-characterized impact of biofumigation on plant-parasitic nematodes, the effects on the non-parasitic part of the community is largely unknown. We investigated the field effects of biofumigation with four Indian mustard (*Brassica juncea*) cultivars on both plant-parasitic and free-living nematodes. Prior to biofumigation, GSL contents of *B. juncea* were determined, and from this, the expected ITC concentrations in the topsoil were calculated. As positive controls, two concentrations of 2-propenyl ITC – corresponding to the average expected ITC concentration, and two times the concentration predicted for the highest producer – were directly applied to wheat (=non *Brassica* control) plots. Although biofumigation resulted in significant changes for most nematode taxa, none of these shifts could be attributed to the release of ITCs. Moreover, none of the two directly applied ITC concentrations resulted in effects on the nematode community distinct from the water control. We therefore conclude that the observed changes in nematode assemblages are related to intense mechanical disturbance, green manure and the absence of host plants for obligatory plant-parasitic nematodes, rather than to the release of ITCs.

1. Introduction

For decades, the use of fumigants in agriculture has been a widespread practice to control soil borne pests (Gamliel et al., 2000). More recently, most synthetic biocides, e.g. chloropicrin and methyl bromide, were placed under strict legislation or entirely banned because of their negative impact on the environment (Gamliel et al., 2000; Ruzo, 2006). These restrictions have created a need for alternative management practices. Biofumigation, i.e., the use of *Brassica* green manures for pest control, is one of these alternatives (Matthiessen and Kirkegaard, 2006). Plants belonging to the Brassicaceae family are known to produce glucosinolates (GSLs), precursors of antifeedants for herbivorous insects. A major group of hydrolysis products of these GSLs, isothiocyanates (ITCs), act as general biocides (Brown and Morra, 1997). Due to the short release time and half-life of GSLs and ITCs in soils (Gimsing and

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Kirkegaard, 2009), direct toxic effects on soil borne pests are expected shortly after biofumigation.

Although biofumigation is often viewed to be less harmful for the environment and soil biota in comparison to synthetic fumigants (Matthiessen and Kirkegaard, 2006), the natural mixture of ITCs can be just as toxic as, or even more toxic than synthetic pesticides (Gimsing and Kirkegaard, 2009). ITCs can affect a broad range of soil organisms, and thus may destabilize soil food webs as has been shown for the synthetic fumigant metam sodium (Cao et al., 2004). There has been considerable interest in the extent to which naturally produced ITCs can simulate the efficacy of common pesticides. Several field studies have shown that the amendment with *Brassica* material can have a suppressive effect on a broad range of soil pathogens (Mojtahedi et al., 1993; Motisi et al., 2009). The efficacy of biofumigation in suppressing plant-parasitic nematodes in field trials has been variable (Ploeg, 2008): results range from high levels of suppression (e.g. Mojtahedi et al., 1993; Rahman and Somers, 2005) to no suppression at all (e.g. Johnson et al., 1992; Stirling and Stirling, 2003).

Within any soil food web, free-living (i.e., non plant-parasitic) nematodes occur at multiple trophic levels, and therefore the impact of biofumigation on these groups is likely to affect soil functioning. Various effects of biofumigation on free-living nematodes have been reported. After biofumigation, Valdes et al. (2012) observed a decrease in plant-parasitic nematodes and an increase of bacterivorous nematodes. Stirling and Stirling (2003) observed only an increase of free-living nematodes, while Gruver et al. (2010) did not record any effect. In the aforementioned studies, effects were assessed weeks after biofumigation, and it is hard to distinguish the direct toxic effects of ITCs from the impact of tillage and or green manure on nematode communities, as these studies did not include GSL measurements.

The objectives of this study were to assess the direct and subsequent effects of biofumigation on nematode communities. Direct effects included toxicity of ITCs and disturbance due to tillage; subsequent effects of biofumigation were related to the amount and quality of the incorporated plant debris. We monitored plant-parasitic nematodes using classic identification and free-living nematodes using a DNA-based method (Vervoort et al., 2012) at the start of the growing season as well as just before and at several time points after biofumigation of four Indian mustard (*Brassica juncea*) cultivars differing in their GSL content. The biomass and GSL content of the plant material was determined prior to incorporation. This approach allowed for an assessment of the impact of different biofumigation-related factors on both plant-parasitic as well as free-living nematodes.

2. Materials and methods

2.1. Study site

The experiment was performed at the field site (52°03′ N, 7°56′ E) of the Julius Kühn-Institut in Münster, Germany, in 2010. In the years preceding our experiment, the field had been cultivated in 2008 with winter wheat (*Triticum aestivum*), in 2009 with winter barley (*Hordeum vulgare*) and in the early spring of 2010 with maize (*Zea mays*). Soil type was a medium loamy sand consisting of 9.2% clay, 13.6% silt and 77.2% sand with 1.3% organic matter and a pH ($CaCl_2$) of 6.4. Nutrient status per 100 g soil at time of planting was 32 mg P_2O_5 (supra optimal according to local agricultural standards), 17 mg K_2O (optimal) and 6 mg Mg (optimal). Total available mineral nitrogen and sulphur were 52 kg N ha^{-1} and 42 kg S ha^{-1}. The experimental plot was prepared on 7 July 2010 by ploughing the remaining maize stubble of the previous crop and applying 292 kg ha^{-1} hydrosulfan (24% N, 6% S, Yara, Dülmen, Germany), i.e., 70 kg N ha^{-1} and 17.5 kg S ha^{-1}, to ensure optimal plant growth.

2.2. Experimental design

Four *B. juncea* cultivars were used: Terrafit, Terratop, Terraplus (P. H. Petersen Saatzucht, Lundsgaard, Germany) and ISCI-99 (Bluformula, Livorno, Italy). The latter cultivar, ISCI-99, was selected as a high GSL producer. As a negative control (a non-GSL crop), wheat (*T. aestivum* cv. Hermann) was chosen. Sinigrin (2-propenyl GSL) is the dominant GSL type in Indian mustard. Its concentration in roots and stems decreases gradually during development, whereas it increases in leaves and reproductive organs of *B. juncea* (Bellostas et al., 2007). At the time of incorporation, the plants were in or just beyond the flowering stage. As a positive control, the ITC derivative of sinigrin, 2-propenyl ITC, was directly applied to the subplots of wheat (see Section 2.4).

A randomized block design with four replicates was used, and the plot size was 4 × 15 m. Based on known germination rates, seeds were sown at densities of 12 kg ha^{-1} for *B. juncea* cvs. Terrafit, Terratop and Terraplus, 15 kg ha^{-1} for *B. juncea* cv. ISCI-99, and 176 kg ha^{-1} for wheat. All plots were drilled on 9 July 2010, hereafter referred to as day 0 (Fig. 1).

2.3. Plant sampling and analysis

Immediately before debris incorporation (day 59), plants were sampled from a 50 × 50 cm subplot within each plot, and root and shoot fresh weights were determined. Aliquots (each ≈ 150 g fresh weight) were collected to determine the respective dry weights (weight loss after 24 h at 70 °C). From each *B. juncea* plot, ten plants were randomly collected, divided into roots and shoots, and all parts were immediately frozen and kept at −80 °C. The material was freeze-dried, pulverized with an oscillating mill (MM2, Retsch, Haan, Germany) and the resulting plant powder was stored for chemical analysis. The GSLs were extracted from a 200 mg subsample using 3 mL methanol:water (70:30, vol/vol) at 75 °C. One mL of GSL extract was loaded on a micro-column filled with DEAE − A25 Sephadex (CAS Number 12609-80-2, Sigma−Aldrich, MO, USA), The extracted GSLs were then converted into desulfo-GSL's by incubation for 16 h at 39 °C with sulfatase from *Helix pomatia* Type H-2 (CAS Number 9016-17-5, Sigma−Aldrich, MO, USA), eluted with H_2O and analysed by High-Performance Liquid Chromatography (HPLC) with Diode-Array Detection (DAD) at a wavelength of 229 nm (Jasco GmbH, Groβ-Umstadt, Germany). GSL quantification was made internal standard-based (more details in Schütze et al., 1999). Finally, GSL yield per hectare was calculated based on GSL concentration of the plant material and plant dry biomass. Carbon and N content of the *B. juncea* material was determined using an elemental analyzer (type EA 1108, Interscience/Carlo Erba, Val de Reuil, France).

2.4. Biofumigation

On day 59, *B. juncea* and *T. aestivum* material was chopped and incorporated into the soil. For this, a tractor-driven flail mower was used, and plant debris was immediately incorporated into the top 20 cm of soil with a rotary tiller. Afterwards, the soil surface was slightly rolled to reduce soil porosity and minimalize evaporation of the ITCs. For the positive control, 2-propenyl ITC was applied directly to the soil. For this purpose, two subplots of 4 m^2 each were selected in each of the four wheat plots. These subplots were treated, after the plant material was chopped and prior to incorporation, with 10 L m^{-2} of a low (1.2 mmol L^{-1}) or a high (4.8 mmol L^{-1}) concentration of 2-propenyl ITC (CAS Number 1476-