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Original article

Relationships between nematode diversity, plant biomass, nutrient cycling and soil suppressiveness in fumigated soils

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A B S T R A C T

Nematodes interact with many other organisms as participants in several links of the soil food web, playing important roles in essential soil processes. Due to their high abundance and diversity of responses to soil disturbance, nematodes are suitable indicators of soil condition. With the aim of inferring soil fumigation effects on agroecosystem functioning, soil nematode diversity, soil properties, plant growth, and soil suppressiveness were monitored in a commercial strawberry farm and its surroundings for two consecutive growing seasons in southern Spain. Our results show that nematode diversity was low in fumigated soils throughout the whole season and, although yearly recovery occurred within the treated fields, fumigated soils showed a permanent perturbed condition. The nematode community was more closely associated to nutrient cycling in non-cropped than in cropped soils, and the link between plant biomass and nematode community structure was weak. Non-treated furrows within the treated fields were a reservoir of both beneficial and plant-parasitic nematodes, but such difference between furrows and beds was not enough to maintain more suppressive soil assemblages in the furrows. Treated soils were less suppressive than unmanaged soils, and there was a positive and significant correlation between soil suppressiveness and soil food web structure and diversity.

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1. Introduction

Spain is the main strawberry (*Fragaria ananassa* Duch.) producer within the European Union [23], with 6.400 ha of cultivation surface, concentrated mostly in Southern Spain (Huelva) [37]. Since the phase out of methyl bromide, producing strawberries has become increasingly challenging, and other alternatives less aggressive to the environment have been used as an alternative to methyl bromide soil disinfection [36]. The use of 1,3-dichloropropene (1,3-D) and chloropicrin (Pic) has increased in strawberry cropping systems, and has become essential to maintain strawberry commercial production in Southern Spain [35,50]. However, these fumigants present uncertain effects on the environment [72] and have been not approved in the European Union [20,21].

Soil is a very important factor in cropping systems; it constitutes a complex aggregate of physical, chemical, and biological components where specialized biota can survive [27], developing important and precise ecological roles [41]. Among soil organisms, nematodes are diverse, abundant and widespread, being present in virtually all habitats across the world, interacting with many other organisms as participants in several links of the soil food web and playing important roles in essential soil processes [29]. Their permeable cuticle permits them to be in contact with dissolved compounds in the soil, and their anatomical features provide information about their feeding roles, making them useful indicators of soil diversity and functioning [19,28,46]. Moreover, nematode classification into ecological groups such as trophic links and the development of soil food web indices [24] have produced a significant advance in the interpretation of the relationships between soil diversity and soil functioning [34,43]. Soil fauna-based indicators must reflect changes in soil web condition as a result of land management practices, and consequently reflect ecological processes [43]. The Scientific Panel of Plant Protection Products and their residues from the European Food Safety Authority (EFSA) has pointed out the necessity of modifying the ecotoxicological data requirements for plant protection products in order to evaluate them in an integrated way, including structural and functional endpoints with organisms such as bacteria, fungi, protozoa and nematodes [58]. The EFSA recommends the use of nematodes

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during the assessment of the functional and structural features of the soil [22].

Soil fauna and soil functioning are closely linked, e.g., the composition and abundance of nematode trophic groups and functional guilds can be related to carbon and nitrogen soil content [4]. Soil fauna primarily affects nutrient cycling by grazing on fungi and bacteria and by excreting N-rich compounds that enrich the soil with nutrients [15]. However, in intensive agricultural systems, such relationships are often missing, since plant nutrient availability does not rely heavily on nutrient mineralization, but instead on mineral fertilizers and organic amendments. Fertilization has a direct positive effect on plant growth, but beneficial fauna such as certain soil nematodes may be sensitive to the disturbance produced by both nutrient enrichment [6,61] and mineral fertilizers [60]. Since different groups of nematodes present different sensitivities to agricultural management and soil disturbance [42], nematodes can be used as indicators of soil food web condition [24], and nematode-based indicators can be used to study the relationships between soil diversity and soil physical–chemical properties, essential to understand soil functioning processes. Nematodes may serve as food source to other soil mesofauna as tardigrades, which occupy a wide range of niches in freshwater and terrestrial environments, some of them possessing a worldwide occurrence. Their ability to undergo cryptobiosis permits them to enter into a latent state in response to desiccation (anhydrobiosis), temperature (cryobiosis), low oxygen (anoxybiosis), and salinity changes (osmobiosis) [47].

Thus soil food webs play essential roles in different ecosystem processes such as plant growth [67], which occurs within a complex environment where both above and below ground interactions among different organisms take place [65]. Previous studies have found significant relationships between plant growth, soil organisms and soil management [9,14]. At the same time, plant identity and diversity closely affect soil biota at different trophic levels [68]. Other soil food web functions, such as soil suppressiveness, may also be important to plant development. Both intrinsic and man-induced soil suppressiveness against a number of disease-causing agents and pest organisms has been repeatedly described in the last 30 years [32,53]. Such suppression of pest organisms can be developed by a number of microorganisms [10], and other organisms such as arthropods [39], tardigrades [56], and nematodes, such as the entomopathogenic Rhabditidae [13]. In agricultural systems, soil management, and especially soil fumigation, may reduce natural soil suppressiveness, turning a suppressive soil into a non-suppressive one [70]. Detrimental agricultural management practices have been associated with unstructured and unhealthy soils with low suppressiveness ability [55], but, in the absence of specific biocontrol agents, the extent to which soil suppressiveness relies on the complexity and diversity of soil food webs has not been completely disentangled.

In previous papers the effects of soil fumigation and the effects of sampling time, habitat, and soil management variation on soil nematode diversity were studied [57]. In this paper we aim to infer if the detected effects on soil diversity have further consequences on soil functioning. Specifically, the objectives of this study were to study the relationship between soil nematode diversity and plant growth, nutrient cycling and soil suppressiveness in fumigated, agricultural soils.

2. Materials and methods

2.1. Study site

The study area was a commercial strawberry farm and its surroundings (including field margins and an adjacent pinewood), located in Cartaya (Huelva, Southern Spain). The soil in the study area had presented a sandy texture in the field and the pinewood, and a clay-loam texture in the field margins. The climate of this area is oceanic with subtropical influence [33], with a mean annual precipitation of 490 mm and a mean annual air temperature of 18.1 °C [1].

Strawberry has been cultivated at the farm for the past 20 years. Although in 1995 the use of methyl bromide was restricted by the Montreal Protocol owing to its ozone-depleting properties [64], this fumigant continue to be used in the farm until 2006 when the prohibition became effective. Since other soil fumigants such as 1,3-dichloropropene (1,3-D; $C_3H_4Cl_2$) and chloropicrin (Pic; CCl_3NO_2) have been proved to be effective MeBr alternatives [50] a commercial mix of 1,3-dichloropropene and chloropicrin and only chloropicrin, have been applied in the farm.

Soil sampling took place in a 1.7 ha section of the commercial farm along two cropping seasons. The farm was managed following conventional standard practices for the area. The farm soil was amended with horse manure at a rate of 20,000 kg ha^{-1} at the beginning of the study, prior to any soil treatment. A commercial mixture of 1,3-D and Pic (Telopic, 1,3-dichloropropene 81.9% p/v, chloropicrin 46.5% p/v) was applied at 400 kg ha^{-1} by injection at 20 cm depth in 2010–2011, and a commercial Pic formulation (Tripicrin, chloropicrin 99%) was applied at 400 kg ha^{-1} in 2011–2012, partially due to the restrictions of 1,3-D use imposed by the European Union [20,21].

Simultaneously with fumigant application, beds were formed and covered with black polyethylene plastic mulch (0.09 μm thick), and plastic irrigation tubing was strategically placed under the cover. The dimensions of the beds and furrows were 42 cm and 45 cm wide respectively. *Honor* strawberry plants were planted at 78,000 plants ha^{-1}, 28 days after fumigation to avoid phytotoxicity. Fertilization occurred through nitrogen–phosphorous–potassium fertigation during the whole length of both cropping seasons. For comparative purposes, field margins and an adjacent pinewood, representing non-cropped soils, were included in the study.

2.2. Soil sampling

Samples were collected from a 1.7 ha section of the strawberry commercial farm, and from its field margins and an adjacent pinewood. Field margins were approximately 3 m wide, and surrounded the 1.7 ha area on three of the field sides. The total length was 450 m. The adjacent pinewood area occupied a surface of 0.25 ha.

Soil samples were collected during two cropping seasons, from September 2010 to June 2012. Soil samples were collected a few days before soil fumigation, after transplantation, at two times during the mid-season, and before the final harvest, corresponding to 5, 17, 28, and 35 weeks after soil treatment (WAT) (September and November 2010, February, April and June 2011) during the growing season 2010–2011, and before soil fumigation and 5, 19, 30, and 39 WAT (September and October 2011, January, April and June 2012) during the season 2011–2012.

Samples were taken with a 5 cm diameter soil corer at 0–20 cm depth along the sampling area. 30 soil samples were taken within the field, five samples were collected from the field margins, and another five samples were collected from the pinewood at each of the five samplings in 2010–2011 (total 200 soil samples). The three habitats (field, field margins, and pinewood soils) were sampled at the same sampling dates. Sampling design was modified in the second cropping season, and soil samples from non-treated furrows between cropping beds were also collected and included in the study. In 2011–2012, 20 samples were collected before soil fumigation, and 20 samples from the beds plus 10 samples from the

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