



Corky root severity, root knot nematode galling and microbial communities in soil, rhizosphere and rhizoplane in organic and conventional greenhouse compartments



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ABSTRACT

Organic and conventional greenhouse compartments were set up to compare tomato production, disease development and microbial communities in two systems side by side. After one year, the organic greenhouse was split into two sections: one where straw was added to the soil (to reduce soil nitrogen) and the other without straw. This paper reports on naturally emerging corky root (CR) disease and root knot nematode (RKN) galling in the second and third years of the experiment. CR increased over the years in all systems, but earlier in the conventional system, so that there were significant differences between the two organic versus the conventional systems in the second year but not anymore in the third year. RKN galling became apparent in the third year, particularly in the conventional system and rarely in the organic systems. CR severity was significantly negatively correlated with dissolved organic carbon content, total carbon, nitrogen concentrations and oxygen uptake rate in soil, and with tomato yield. RKN galling was too rare to be related to any variables. CR severity classes were significantly separated based on selected operational taxonomic units (OTUs) or phylotypes of 16S rDNA for bacteria and actinomycetes and of 18S rDNA ITS for fungi detected by denaturing gradient gel electrophoresis (DGGE). Microbial communities in the rhizoplane were largely subsets of those in the rhizosphere and bulk soil, but the distributions of OTUs over different habitats were similar for the conventional and recently converted organic systems. These results may stimulate research into selection of microbial communities from one habitat into neighboring ones to support the hypothesis of microbial cycling in ecosystems.

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1. Introduction

Organic farming has increased considerably in importance world-wide over the past 20 years with growth rates over 10 % per year in many countries (Willer and Lernoud, 2015). This is also true for the production of greenhouse vegetables, although the proportion of organically produced greenhouse crops is still very small (<1%). In Europe, organic greenhouse production must take place in natural soil, while organic greenhouse crops can be produced in potting mixes in Canada (Canada General Standards Board, 2006) and the USA (van Bruggen, 2015). In all cases, synthetic pesticides and inorganic fertilizers cannot be used.

Various organic amendments are applied in organic systems to maintain soil structure and fertility, and crop rotations are generally longer in organic than in conventional arable crop production (Leoni et al., 2015). As a consequence, root diseases are generally less severe in established organic farms than in conventional farms (van Bruggen and Termorshuizen, 2003; van Bruggen and Semenov, 2015; van Bruggen et al., 2015a). However, if a cash crop is planted too soon after organic amendment, the cash crop may succumb to seedling damping-off caused by various pathogens like *Pythium* and *Rhizoctonia* (Grünwald et al., 2000; He et al., 2010; Zelenev et al., 2000, 2005). Nevertheless, most other root diseases and some nematode pests are suppressed in organically farmed soils (van Bruggen and Termorshuizen, 2003; van Bruggen and Semenov, 2015). For example, *Fusarium* root rot (*F. graminearum*) and take-all disease (*Gaeumannomyces graminis*) were reduced in organic compared to conventional wheat fields (Hiddink et al., 2005; van Bruggen and Termorshuizen, 2003).

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Corky root (*Pyrenochaeta lycopersici*) and Phytophthora root rot (*P. parasitica*) of tomato were also less severe in organic than in conventional fields (Clark et al., 1998a; Workneh et al., 1993). In addition, populations of the lesion nematode *Pratylenchus* spp. were lower in organically than in conventionally managed soils, and juveniles of *Meloidogyne javanica* were more suppressed in bioassays with organically compared to conventionally managed soil (Berkelmans et al., 2003). However, the suppression of *Pratylenchus* and *Meloidogyne* populations was dependent on the type of organic material incorporated into soil (Briar et al., 2007; Everts et al., 2006; McSorley, 2011; Thoden et al., 2011).

Various reasons for the suppression of root diseases in organically managed fields have been proposed. Positive correlations have been found between the severity of various diseases and soil or plant nitrogen concentrations (van Bruggen et al., 2015a,b; Workneh and van Bruggen, 1994b), which are generally lower in organically managed field soils (Clark et al., 1998b; van Diepeningen et al., 2006). Negative correlations have been found between disease severity and soil pH, which is often lower when inorganic nitrogen fertilizers are used (Clark et al., 1998b; Mäder et al., 2002; Senechkin et al., 2014). The most significant and consistent relationships have been documented between root disease suppression and microbial activity and biomass, which are commonly higher in organically than in conventionally managed soils (Fließbach et al., 2007; Grünwald et al., 2000; Mäder et al., 2002; Workneh and van Bruggen, 1994b). In some cases, positive relationships have been found between root disease suppression and microbial or microfaunal diversity (Workneh and van Bruggen, 1994a), which are also generally higher in organically than conventionally managed soils (Drinkwater et al., 1995; Mäder et al., 2002; van Diepeningen et al., 2006).

Highly diverse ecosystems with numerous taxa that form a complex food web with many trophic levels are generally considered to be healthy, thriving ecosystems (Duffy et al., 2005; van Bruggen and Semenov, 1999, 2000). Therefore, taxonomic and functional diversity indices, determined by various DNA-based methods and physiological tests, are often used as indicators for the health status of soils (van Bruggen et al., 2006; van Diepeningen et al., 2006). Although soils with a higher biological diversity and activity are frequently more suppressive to root-infecting fungi or bacteria than biologically impoverished soils (Hiddink et al., 2005; Messiha et al., 2009; van Bruggen et al., 2015a; Workneh and van Bruggen, 1994a, 1994b), the relationships between microbial communities in soil, rhizosphere, rhizoplane and root disease suppression have not been investigated extensively. In one study, the differences in bacterial communities between the rhizosphere of wheat and adjacent bulk soil differed more in conventionally than organically managed soil (van Diepeningen et al., 2005). Thus the rhizosphere effect was greater in the former than in the latter soil, suggesting that root exudates were utilized more quickly close to the root surface in organically managed soils. Nevertheless, it is not well known to which extent the microbial compositions in the rhizoplane and rhizosphere, where pathogen suppression presumably takes place, reflect the composition in the bulk soil, which is known to differ between organically and conventionally managed fields (van Diepeningen et al., 2006).

Despite the reduction in root diseases generally observed in organically managed fields, very little is known about root disease suppression in organic greenhouse soils (van Bruggen et al., 2015b). Because almost exclusively, highly valuable vegetable crops are grown in greenhouses, such as indeterminate tomatoes, bell peppers and cucumbers, crop rotation is usually very narrow, also in organic greenhouses. Such a narrow rotation may lead to a build-up of populations of fungal pathogens and phytophagous nematodes (van Bruggen, 2015). For example, corky root

(*P. lycopersici*), Verticillium wilt (*V. dahliae*) and root knot nematodes (*Meloidogyne* spp.) can become really problematic in organic greenhouses (Giotis et al., 2009; Hasna et al., 2009; Klein et al., 2012; Minuto et al., 2006; van Bruggen, 2015; Vitale et al., 2011). It has been difficult to manage these diseases under limited rotation possibilities in those greenhouses (Giotis et al., 2009; Hasna et al., 2007, 2009; Varela et al., 2009).

A greenhouse experiment was set up at Wageningen University in 2003 to compare tomato production and disease severity in organically and conventionally managed greenhouse compartments (Gravel et al., 2010). In bioassays with the different greenhouse soils in 2004 and 2005, suppression of Fusarium wilt of flax was greater in soil from the organic compartment than in that from the conventional compartment (van Bruggen et al., 2015b). This suppression was related to soluble carbon concentrations, various bacterial measurements, microbial activity and nitrate concentrations. However, thus far, the occurrence and severity of root diseases on the tomato plants in this experiment have not been published. Microbial community analyses were published for the organic and conventional bulk soils (van Bruggen et al., 2015b), while root disease severity is likely more affected by microorganisms in the rhizosphere and rhizoplane than in the bulk soil. The relationships between the microbial communities and these different habitats have not been published either.

The main aim of this study was to investigate if various soil chemical and biological characteristics, in particular the diversity and composition of the bacteria, actinomycete and fungal communities in soil, rhizosphere and/or rhizoplane, were related to the severity of corky root (*P. lycopersici*) and root knot nematodes (*Meloidogyne incognita*) on tomatoes grown in organic and conventional greenhouse compartments. An additional aim was to investigate to which extent the microbial communities in the rhizoplane were subsets of those in the rhizosphere and soil, and differed between the organic and conventional systems.

2. Materials and Methods

2.1. Greenhouse experiment.

In the Fall of 2003 an experiment was set up at Wageningen University, the Netherlands, to compare tomato production in an organic soil-bound system with that in a conventional soil-bound system in two identical greenhouse compartments of 150 m² each (Gravel et al., 2010). At the start of the experiment, the sandy soil had the following characteristics: pH_{KCl} 5.7; organic matter content 9.3%; clay content (particles < 2 µm) 8%; CaCO₃ 0.2%. In both greenhouse compartments, the soil was rototilled to a depth of 25 cm. To supply sufficient nutrients for the high tomato yields commonly attained in Dutch greenhouses (300–500 mT per ha over a 9-month period), large inputs were needed in both greenhouse compartments. In the Fall of 2003, the organic compartment received 153 tons ha⁻¹ of composted green waste and 109 tons ha⁻¹ of partially decomposed cow manure, which was incorporated into the soil to a depth of 15 cm (Fig. S1A and B). Subsequently a mixture of common vetch (*Vicia sativa*) and rye (*Secale cereale*) was sown and turned into the soil three months later.

As the soil nitrogen content was very high in the organic compartment (Gravel et al., 2010), this compartment was split into two sub-compartments in January 2005, named organic1 (ORG1) and organic2 (ORG2). In both organic sub-compartments, composted green waste (157 tons ha⁻¹) and cow manure (27.3 tons ha⁻¹) were applied in the Winter of 2005. In addition, straw was incorporated into the soil of ORG2 (200 kg straw in 75 m²). Similar amounts of organic materials were added in Winter 2006 (Gravel et al., 2010). The conventional greenhouse did not receive any

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