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The impact of legume and cereal cover crops on rhizosphere microbial communities of subsequent vegetable crops for contrasting crop decline

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HIGHLIGHTS

- The impact of two cover crops on next vegetable growth was investigated.
- Hairy vetch and barley harbored differing root colonizing fungal communities.
- Hairy vetch and barley did not affect differently root fungi in following crops.
- Tomato and zucchini showed the best growth response after hairy vetch.
- Vetch and barley induced a diverse rhizosphere bacterial composition in vegetables.

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ABSTRACT

Crop decline is a progressive soil health reduction commonly associated to the specialized cultivations. As a series of biotic factors are involved in this phenomenon, it can be controlled with essentially agronomic methods, the impact of winter cover crops on early growth at subsequent vegetable crops was investigated in a two-year field study. Barley and hairy vetch were incorporated into the soil following two mechanical terminations of cover crops (green manure and green mulching). Immediately after, tomato and zucchini seedlings were transplanted and grown for 28 days. The above-ground biomass of the vegetables was taken as indicator of crop response to pre-plant treatments, whilst root colonizing fungi and rhizosphere bacteria were the two analyzed soil microbial components. Root-colonizing fungi were evaluated using culture-based methods, bacteria were analyzed by amplification of rhizosphere soil DNA with 16S rDNA, then processed with PCR-DGGE. Tomato and zucchini growth response after vetch was always significantly higher than after barley regardless of mechanical termination. Rhizosphere bacterial communities differed significantly between cover crops and this differences was maintained also in the subsequent vegetable crops. Root-colonizing fungi differed between barley and vetch, although they shared most species (70%) such as *Pythium* spp., binucleate *Rhizoctonia* AG-A and several *Fusarium* spp. The latter three fungal groups were also found most abundant in tomato and zucchini roots. Pathogenicity test showed that tomato was more susceptible than zucchini to *Pythium*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium* spp. and that their relationship with vegetable plants varied from pathogenic to neutral, up to mutualistic in the case of *Rhizoctonia* AG-A. These findings indicate difficulty to identify specific biotic agents responsible of crop decline. Tomato and zucchini showed an undoubted growth improvement after legume; however, a certain specificity of *Cylindrocarpon*-like fungi and *Phoma* spp. to hairy vetch suggests that, when exploiting benefit of this cover crop for mitigating yield decline of vegetable crops, legume should alternate cereal and other botanically distant genera in rotation.

1. Introduction

Soil-borne fungal pathogens can cause yield losses in herbaceous and tree fruit crops and forest trees; but vegetable crops are commonly considered the most susceptible to those pathogens, especially in the early stages of growth (Koike et al., 2006). Yield losses due to soil borne diseases have been estimated accounting for 10% in vegetable crops in 2009 in U.S. (Sherm, 2012), however, up to 60% yield losses have been recorded in specific crops such onion (Schwartz, 2011). The biotic causal agents of crop yield decline are difficult to estimate because the resulting lack of vigor

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and, later, poor yields may be mistakenly ascribed to abiotic stress (cold, water stress) or insufficient plant nutrition rather than the presence of root disease (Laemmlen, 2001).

Vegetable and fruit tree crops, which account for 10.7% of the organic area in EU-15 (the European Union of 15 member states before 1 May 2004), are higher in the organic sector than in EU agriculture as a whole, given that the demand for fruit and vegetables for the organic market is the highest (Stephen and Willer, 2014). Pest and pathogen control in organic agriculture is mainly based on prevention with cropping practices aimed at controlling through the suppression mechanisms (Bugg and Pickett, 1998).

Italy, is the EU member state with the largest area invested with organic vegetable (almost 23,405 ha) followed by Germany (18,000 ha), France and Spain. Theoretically, an organic cropping system should have the capacity to suppress root diseases; however, organic vegetable farms in Italy are mostly located near urban areas and are small-medium sized, thus produce vegetables in rotation on the same land. In this context, such cropping systems are subject to a gradual increase of soil-borne pathogens and a decrease of microbial balance which are responsible of progressive growth reduction generally defined as "crop decline". (Pérez-Brandán et al., 2014a). The complex of soil borne fungal pathogens causing crop decline varies according to the agro-environment and the target crops; moreover, crop susceptibility decreases from early to ripening growth stages (Jarosz and Davelos, 1995). The uneven distribution of soil-borne fungal pathogens across fields and difficult in diagnosis make overall difficult a direct correlation with crop losses (Dixon and Tilston, 2010). For this reason, plant growth parameters such as plant biomass are commonly used as indicator of disease severity or soil health (Johnson, 1993; Gram et al., 2016).

One of the most frequent agronomic practices adopted to mitigate crop decline in organic farms devoted to vegetable production of Central and Northern Italy, is the enrichment of crop sequence with winter cover crops, which improves functional diversity (Lamb et al., 2011), reduces soil erosion, preserves soil organic matter content and soil biomass, increases nutrient supply and N leaching control (Struik and Bonciarelli, 1997).

Barley (*Hordeum vulgare* L.) and hairy vetch (*Vicia villosa* Rhot.) are the most commonly adopted winter cover crops in Italy and worldwide. However, unlike to their impact on nutrient supply and soil erosion N which has been largely investigated (Cicek et al., 2014), little is known on how they can influence rhizosphere microbial communities and any associated effect on the crop health. Therefore, a two-year study on plant-soil microbial interaction was performed in an organic experimental field for vegetable production in central Italy to evaluate whether barley and hairy vetch can affect rhizospheric bacteria and root-colonizing fungi of subsequent vegetable crops.

Two different mechanical termination methods for incorporating cover crops into the soil, were added to the experimental set. Indeed, cropping practices have a great impact in organic agriculture, where plant residues turnover guarantees most of nutrient supply.

2. Material and methods

2.1. Experimental site description

A two-year trial was carried out in a long term organic experimental field. The study was limited to the growing period immediately after transplant which represents one the most critical stages of plants in horticulture due to the high susceptibility of seedlings to soil-borne pathogens.

The experimental field was located near Monsanpolo del Tronto, in the coastal area of the Marche Region in Central Italy (latitude 42° 53′ N, longitude 13° 48′ E) with Mediterranean climate. The soil taxonomy classification was Fine-loamy mixed thermic. Soil organic matter content accounted for 1.6% with a pH 8.2 in the experimental field at the beginning of this trial in spring 2014. This field was under organic management for 14 years; during this period, six vegetable crops (tomato, zucchini, fennel, melon, cauliflower and bean/lentil) were cultivated over a 4-year rotation. Plant vigor decline in post plant period of tomato and zucchini was observed in the last years; moreover, tomato and zucchini gave an variable performance in the last 6 years, indicating a non-specific crop decline.

2.2. Field trial description

The impact of two winter cover crops (barley and hairy vetch) and two termination techniques (green manure and green mulching obtained by flattening plants with roller crimper) was evaluated on two subsequent vegetable crops: tomato and zucchini. The same factorial experiment was repeated in 2014 and 2015 (Fig. 1).

Barley cv. Trasimeno and hairy vetch cv. Minnie were sown in late October and late September respectively on two main plots

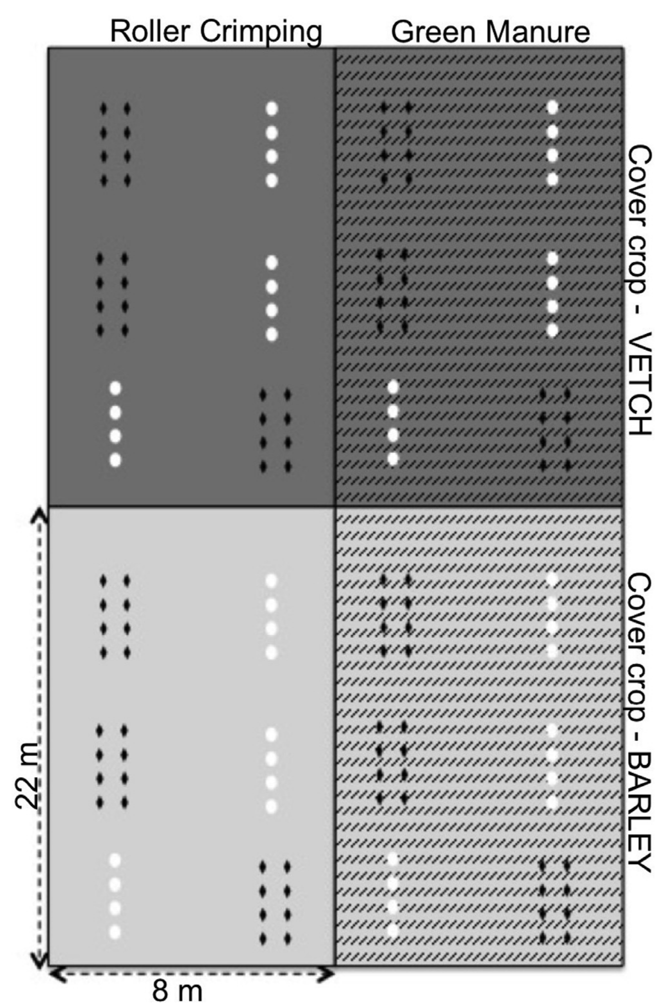

Fig. 1. Map of experimental design where tomato and zucchini growth response to 2 previous cover crops (hairy vetch and barley) and 2 techniques of cover crop termination (green manure and roller crimping) were evaluated. The same experimental design was two years repeated. ○ Zucchini; ◆ Tomato.

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