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Impact of a pesticide cocktail (fenhexamid, folpel, deltamethrin) on the abundance of Glomeromycota in two agricultural soils

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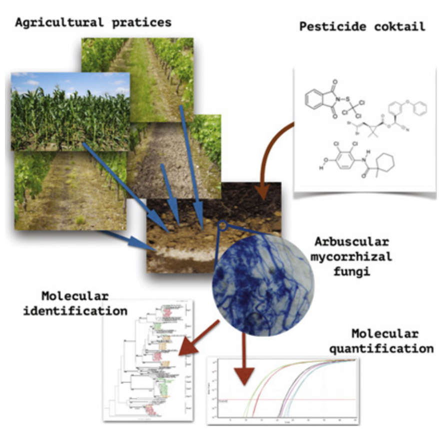
HIGHLIGHTS

- Studied soils differed in their history and biological characteristics.
- Mycorrhizal fungal infectivity and diversity was low in vineyard soils.
- A pesticide cocktail affected Glomeromycota populations in soil.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticide contamination of the environment can result from agricultural practices. Persistence of pesticide residues is a threat to the soil biota including plant roots and beneficial microorganisms, which support an important number of soil ecosystem services. Arbuscular mycorrhizal fungi (AMF) are key symbiotic microorganisms contributing to plant nutrition. In the present study, we assessed whether AMF could indicate eventual side effects of pesticides when directly applied to field soils. We evaluated the ecotoxicological impact of a cocktail of three commonly used agricultural pesticides (fenhexamid, folpel, deltamethrin) on the abundance and composition of the AMF community in vineyard (Montagne de Saint-Emilion) and arable (Martincourt) soils subjected to different agricultural practices. The dissipation of applied pesticides was monitored by multiresidual analyses to determine the scenario of exposure of the AMF community. Diversity analysis before application of the pesticide cocktail showed that the AMF communities of vineyard soils, subjected to mechanical weeding or grass cover, and of the arable soil subjected to intensive agriculture, were dominated by Glomerales. Ribotypes specific to each soil and to each agricultural practice in the same soil were found, with the highest abundance and diversity of AMF being observed in the vineyard soil with a grass-cover. The abundance of the global AMF community (Glomeromycota) and of three taxa of AMF (*Funneliformis mosseae*, *Claroideoglomus etunicatum*/*C. claroideum*)

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was evaluated after pesticide application. The abundance of Glomeromycota decreased in both soils after pesticide application while the abundance of *Claroideoglomus* and *F. mosseae* decreased only in the arable soil. These results show that higher doses of pesticide exposure did not affect the global abundance, but altered the composition, of the AMF community. Resilience of the AMF community composition was observed only in the vineyard soil, where *F. mosseae* was the most tolerant taxon to pesticide exposure.

1. Introduction

After application to crops, pesticides and their main metabolites can persist in agricultural soils from where they can be transferred by various processes to other compartments of the agrosystem (e.g. water resources). In addition, these chemical residues can represent an ecotoxicological risk to soil organisms that support ecosystem services. The microbial community is a tremendously diverse component of the soil biota, abundant and actively contributing to a range of ecosystem functions. Recently, the EFSA (European Food Safety Agency) panel for Plant Protection Products and their Residues (PPR, 2010) recognized soil microbes as one of the seven key drivers for those ecosystem services that are potentially impacted by pesticides in agricultural landscapes. It proposed, as a specific protection goal for microbes, to monitor functional groups to fulfill the environmental risk assessment of pesticides, comforting the assumption that soil microorganisms are potential indicators of biological quality in agroecosystems (Roper et al., 1997).

Arbuscular mycorrhizal fungi (AMF), an important component of the functional groups of soil microorganisms, belong to the phylum Glomeromycota and are ubiquitous in terrestrial ecosystems. They are obligate symbionts forming the arbuscular mycorrhiza (AM) symbiosis with roots of the vast majority of the vascular plants (Smith and Read, 2008), including many agricultural and horticultural crops. Because of their role in supplying poorly mobile nutrients (e.g. phosphorus), to plants (Parniske, 2008), as well as increasing tolerance to abiotic (e.g. drought) and biotic (i.e. pests) stresses (Kiers et al., 2011), AMF are a functional group contributing to plant growth and health to be preserved to ensure the sustainability of the agrosystems. Given the fact that modern agriculture relies on the generalized use of fertilizers and pesticides, AMF are exposed to diverse chemicals. If the negative effect of phosphate fertilizers has already been shown on AMF (Smith et al., 2011), much less is known regarding their interaction with pesticides. Several studies have shown that AMF can respond in different ways to pesticide exposure depending on pesticide classes. Fungicides such as fenhexamid and fenpropimorph, designed to eradicate fungal pests, have clear negative effects on AMF (Zocco et al., 2008, 2011). Using an in vitro test on Ri T-DNA-transformed carrot roots colonized by the AMF *Rhizophagus irregularis*, these authors showed that high concentrations of fenhexamid caused a significant decrease in fungal spore germination, germ tube elongation, extraradical mycelium architecture and spore formation (Zocco et al., 2008), as well as in phosphorus (P) transport and alkaline phosphatase and succinate dehydrogenase activities associated with the extraradical mycelium (Zocco et al., 2011). Furthermore, using the same in vitro test, Campagnac et al. (2008) reported that fenhexamid did not modify sterol profiles and total percentage of AMF colonization of transformed carrot roots, but reduced significantly the arbuscule frequency. In contrast, folpel, another fungicide, did not affect colonization by AMF in *Cynara cardunculus* (Marin et al., 2002). Insecticides, not designed to target fungal species, seem to have no inhibitory effect on AMF (Nemec et al., 1981; Schweiger and Jakobsen, 1998), or can even stimulate AMF root colonization and P uptake (Spokes et al., 1981). In addition AMF have been shown to overcome the negative impact caused by the insecticide pyrethroid bifenthrin on the growth of corn (Corkidi et al., 2009). Finally, herbicides can impact AMF either directly (Li et al., 2013; Pasaribu et al., 2011), or indirectly by destroying their host plants (Druille et al., 2013). All these studies suggest that AMF are responsive to pesticide exposure. However, up to now, the vast majority of these investigations assessing pesticides impact on AM interactions have been carried out by tests either in vitro or in pot cultures by considering plant growth, AMF root colonization and/or P uptake as endpoints to estimate the potential toxicity of pesticides. Consequently, these tests are not providing any information on the ecotoxicological impact of pesticides on the biodiversity of AMF supporting AM symbiotic interactions. Despite the fast development during the last two decades of molecular tools to study the abundance, the structure and diversity of AMF communities very little research has used them to analyze the ecotoxicological impact of pesticides on the abundance, structure and diversity of AMF communities in soils. A recent study reported that *Glomus* sp. and *Funneliformis mosseae*, members of the indigenous AMF community in a field study, were affected by the ergosterol biosynthesis inhibitor carbendazim, at 40 days post-application (Ipsilantis et al., 2012). More recently, the negative impact of field-applied nicosulfuron, a sulfonylurea herbicide, on the abundance and diversity of AMF was shown (Karpouzas et al., 2014).

In the present study, we assessed whether AMF could indicate eventual side effects of pesticides when directly applied to field soils. Considering the scarcity of information on pesticide impacts on AMF communities, we combined the use of molecular tools with conventional methods to study the ecotoxicological impact of pesticides on the abundance and diversity of AMF in soils. For this, we chose to use soil microcosms incubated under controlled conditions. Given the fact that France is an important wine producer (Bouffaud et al., 2015), and that the culture of *Vitis vinifera* mainly depends on the application of plant protection products like fungicides and insecticides, we chose the scenario of exposure of a cocktail of pesticides to the soil microcosms (two fungicides: fenhexamid, folpel, and one insecticide: deltamethrin) that are representative of yearly pesticides applications in vineyards. Two soil types from under different agricultural practices were compared: (i) a vineyard soil from Montagne de Saint-Emilion (Bordeaux) continuously cropped according to one of three different modalities to control weeds (mechanical weeding, grass cover or chemical weeding) and (ii) an arable soil from Martincourt (Meurthe-en-Moselle) cropped with a wheat/rapeseed rotation. Evolution of the abundance of AMF populations was monitored molecularly throughout incubation of the soils with the pesticide cocktail using nested and real-time PCR on purified DNA extracted directly from soil samples.

2. Materials and methods

2.1. Soils

Soil was collected from two locations: Montagne de Saint-Emilion (Bordeaux vineyard) and Martincourt (experimental farm of INRA Martincourt) in France (Table 1). The soil of Montagne de Saint-Emilion was cropped with grapevines according to three different agricultural practices to control weed development: mechanical weeding (SE-MW), grass cover (SE-GC), and chemical weeding (SE-CW). The soil of Martincourt (M-CA) was subjected to intensive agricultural practices and cropped with a wheat/rapeseed rotation. Soil was sampled from each site according to ISO 10381-6 recommendations (Soil quality – Sampling – Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory). Briefly a composite sample of the Ap horizon (0–20 cm) made of at least