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Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide

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ABSTRACT

Copper-based fungicides have been applied in apple orchards for a long time, which has resulted in increasing soil Cu concentration. However, the microbial and enzyme properties of the orchard soils remain poorly understood. This study aimed to evaluate the effect of long-term application of Cu-based fungicides on soil microbial (microbial biomass carbon (C_{mic}), C mineralization, and specific respiration rate) and enzyme (urease, acid phosphatase, and invertase activities) properties in apple orchards. Soil samples studied were collected from apple orchards 5, 15, 20, 30, and 45 years old, and one adjacent forest soil as for reference. The mean Cu concentrations of orchard soils significantly increased with increasing orchard ages ranging from 21.8 to 141 mg kg^{-1}, and the $CaCl_2$-extractable soil Cu concentrations varied from 0.00 to 4.26 mg kg^{-1}. The soil mean C_{mic} values varied from 43.6 to 116 mg kg^{-1} in the orchard soils, and were lower than the value of the reference soil (144 mg kg^{-1}). The ratio of soil C_{mic} to total organic C (C_{org}) increased from 8.10 to 18.3 mg C_{mic} g^{-1} C_{org} with decreasing orchard ages, and was 26.1 mg C_{mic} g^{-1} C_{org} for the reference soil. A significant correlation was observed between total- or $CaCl_2$-extractable soil Cu and soil C_{mic} or C_{mic}/C_{org}, suggesting that the soil Cu was responsible for the significant reductions in C_{mic} and C_{mic}/C_{org}. The three enzyme activity assays also showed the similar phenomena, and declined with the increasing orchard ages. The mean soil C mineralization rates were elevated from 110 to 150 mg CO_2–C kg^{-1} soil d^{-1} compared with the reference soil (80 mg CO_2–C kg^{-1} soil d^{-1}), and the mean specific respiration rate of the reference soil (0.63 mg CO_2–C mg^{-1} biomass C d^{-1}) was significantly smaller than the orchard soils from 1.19 to 3.55 mg CO_2–C mg^{-1} biomass C d^{-1}. The soil C mineralization rate and the specific respiration rate can be well explained by the $CaCl_2$-extractable soil Cu. Thus, the long-term application of copper-based fungicides has shown adverse effects on soil microbial and enzyme properties.

1. Introduction

Copper-based fungicides have been recognized for more than two centuries (Pietrzak and McPhail, 2004). The mixtures of Cu sulphate and lime (Bordeaux mixture) have been widely used in pome and stone fruit orchards, vineyards and vegetable crops to control fungal diseases for over 100 years (Merry et al., 1983). However, foliar application of these fungicides leads to a significant input of Cu to soil, through direct application, drift, or dripping of excess sprays from leaf surfaces (Chaignon et al., 2003). Numerous studies indicated that long-term use of Cu-based chemicals resulted in increased soil Cu concentrations, e.g., 100–1500 mg kg^{-1} in France (Besnard et al., 1999); 29–131 mg kg^{-1} in India (Prasad et al., 1984); and 11–320 mg kg^{-1} in Australia (Wightwick et al., 2008). Consequently, some European countries have introduced restrictions on the use of Cu fungicides to protect the environment, which also satisfy eco-labelling requirements. For example, the Netherlands has banned Cu fungicides, while Switzerland has restricted the amount of Cu that can be applied per hectare (Gardner, 2003).

Up to now, most studies are limited to the total soil Cu concentration and its distribution among soil components in the orchards receiving long-term application of Cu fungicides (Arias et al., 2004; Fernandez-Calvino et al., 2008). As fungicides are applied to control fungal diseases, they will also affect beneficial soil fungi and other soil organisms. Therefore, in many regions of the world there are increasing concerns that Cu may reach the concentrations in orchard soils toxic to soil organisms, or even phytotoxicity (Brun et al., 2001; Viti et al., 2008).

Although the total soil Cu concentration is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact. Since biological

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properties are sensitive to change, they are being considered to be suitable indicators of environmental impact for properly complementing the soil physicochemical properties (Bending et al., 2004). Brookes (1995) found that there were a number of basic criteria that a microbiological property might be expected to fulfill as an indicator in monitoring soil pollution by metals or other pollutants. It is preferable that these properties can be easily and economically measured; these properties need to be sensitive enough to indicate pollution but also sufficiently robust not to give false alarms; it may be that reliance upon a single property is unsafe. Thus, measurements of the status and activity of specific organism contributing to soil processes have the potential to provide rapid and sensitive means characterizing soil quality. Soil microbial biomass is considered to be a transformation agent of soil organic materials and a labile pool for plant nutrients. Hence, the change of the soil microbial biomass could lead to a change in the rate of nutrient cycling and the size of the nutrient pool. Brookes (1995) suggested that soil C mineralization and linked parameters such as CO_2–C production per unit biomass C and unit time (biomass specific respiration rate) might be useful as indicators for the change of soil function. Soil enzyme activities are also useful for detecting changes in soil quality, as they underpin nutrient cycling, and also function as signals of altered microbial community structure caused by environmental impact (Kandeler et al., 1996).

In China, commercial apple orchards are highly manipulated agro-ecosystems, where a conventional protection strategy has input a large amount of Cu-based fungicides (Bordeaux mixture) to protect apple trees from serious fungal attacks since the trees are planted. However, by now, no guidelines are established in China for the sustainable management of Cu-based fungicides in orchards. In addition, to our knowledge, very little is known about the shifts in soil biota due to apple orchard management practices that result in an accumulation of high levels of Cu in soils derived from Cu-based fungicides. Especially, information relating to non-target effects of these fungicides on total soil microbial biomass, microbial activity and soil enzymes is lacking. The objectives of this study were to investigate the change of soil Cu concentrations in apple orchards with different ages, measure microbial and enzyme responses of soil Cu pollution, and evaluate the potential risks of long-term application of Cu fungicides in orchards.

2. Materials and methods

2.1. Study sites and experimental design

The research area is located at Rizhao City (35°04′–36°04′N, 118°25′–119°39′E), the southeast of Shandong Province, China. The annual average temperature and precipitation of this region are 12.7 °C and 1147 mm, respectively. The soil type is brown soils (*Udic Luvisols*), and the clay content (<2 μm) of the area studied varies from 128 to 131 g kg^{-1}. Because the orchards studied are joint properties belonging to a village, they received the same amounts of Cu-based fungicide (Bordeaux mixture, 16 kg ha^{-1} Cu applications per year, the ratio of $CuSO_4$:CaO:H_2O is 1:2:250) and fertilizer (poultry and swine manure, about 100 kg per tree per year). The Cu-based fungicides were applied right from the beginning of the apple trees being planted for 4–5 times per year.

The soil samples were collected from apple orchards 5, 15, 20, 30, and 45 years old in May 2008. Six soil samples were collected from each orchard, and each sample was a composite of six sub-samples. The soil sub-samples were collected at a distance of 1 m from apple tree trunk at a depth of 0–20 cm (after removing the litter layer). An adjacent forest (Pine) which had not received any artificial input of copper (native soil) about 1 km away from the orchards was chosen to collect a reference soil.

Part of each soil sample was air-dried at ambient temperature, crushed, and sieved to pass through a 2 mm sieve to analyze soil pH and other physicochemical properties. The remaining soil was kept moist in the dark at 4 °C to assess soil C_{mic}, soil C mineralization rate, and soil enzyme activities.

2.2. Determination of soil physicochemical properties

The soil samples were ground to pass through a 100-mesh screen (Φ 0.149 mm), and then digested with HF–HNO_3–$HClO_4$ for determination of total soil Cu concentrations by Inductively Coupled Plasma (ICP) with detection limit of 8 μg L^{-1}. Soil pH was measured by a pH meter with a ratio of 1:2.5 soil to water. Soil water holding capacity was determined according to Hillel (1998). Available soil P, K, and N concentrations were determined according to Bray and Kurtz (1945), Jones (1973), and Nelson and Bremner (1972), respectively. Soil C_{org} was determined by oxidizing a soil solution with $K_2Cr_2O_7$ and concentrated H_2SO_4 at 170–185 °C, and then titrating the solution with $FeSO_4$. The $CaCl_2$-extractable soil Cu concentrations were determined by ICP after shaking 5.0 g of soil with 25 ml of 10 mM $CaCl_2$ solution in an end-over-end shaker for 2 h, centrifuging the solution for 10 min at 3000 r min^{-1} and filtering it through a Whatman No. 1 filter paper.

2.3. Soil microbial biomass carbon

Soil C_{mic} was determined by the fumigation extraction method (Vance et al., 1987). The soil C_{mic} was calculated according to the equation: $C_{mic} = E_C/K_{EC}$ (Vance et al., 1987), where E_C was the difference between the extractable C from fumigated and non-fumigated samples, and $K_{EC} = 0.38$ (Ocio and Brookes, 1990). Carbon in the extracts was determined immediately after extraction using an automated total organic C analyzer (Multi N/C 3000, Analytic Jena, Germany). The ratio of C_{mic} to C_{org} was calculated for all soil samples (Anderson and Domsch, 1989) and expressed as mg C_{mic} g^{-1} C_{org}.

2.4. Soil enzyme assays

The criteria for the selection which enzyme activities were assayed were their relative importance in soil nutrient cycling and the simplicity of the assays. Soil urease activity was measured following a method developed by Guan (1986). This method involved the incubation of the soils with added 10 ml of 10% urea, 0.5 ml toluene and 20 ml citrate buffer (pH 6.7) at 37 °C for 24 h. The resulting suspensions were filtered. Then, phenol and sodium hypochlorite were added. For calibration, seven standards were prepared (0.5, 1, 3, 5, 7, 10, and 13 mg NH_4–N ml^{-1}). The blue-colored complexes were measured within 1 h using spectrophotometer as for urease activity at $\lambda = 578$ nm after a 30-min color development period.

For the measurement of soil acid phosphatase activity (Garzillo et al., 1996), soil (5 g) was incubated with 20 ml of 0.5% disodium phenyl phosphate of acetate buffer (pH 5.0) at 37 °C for 2 h. The phenol produced was extracted and oxidized by potassium hexacyanoferrate in alkaline solution. The oxidation products were determined with 4-aminoantipyrine colorimetric method at 510 nm. Assays without soil and without disodium phenyl phosphate were examined as controls at the same time.

The invertase activity was measured following the modified method by Ohshima et al. (2007), and 15 ml of 8% sucrose was used as substrate. Soil portions (5 g) were incubated with 15 ml of 8% sucrose, 0.5 ml toluene and 5.0 ml phosphate buffer (pH 5.5) at