Geoderma 316 (2018) 70–77

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Changes in soil phenol oxidase activities due to long-term application of compost and mineral N in a walnut orchard

Martina Mazzon, Luciano Cavani*, Alja Margon, Giovambattista Sorrenti, Claudio Ciavatta, Claudio Marzadori

Department of Agricultural Sciences, Alma Mater Studiorum University of Bologna, Viale Fanin 40, 40127 Bologna, (Italy)

ARTICLE INFO

Keywords:
Fertilization management
Soil depth
Specific enzyme activity
Soil fertility
Phenol oxidase activity
Soil organic matter turnover

ABSTRACT

Phenol oxidases (POs) are a group of soil extracellular oxidoreductase enzymes, which are involved in oxidative processes related to nutrient cycling. This class of enzymes has multiple functions at both the organism and ecosystem level and can trigger either positive or negative feedback loops between soil organisms and soil organic matter.

The purpose of this study was to evaluate: (i) whether PO activities have a trend different from those of microbial biomass and activity; and (ii) whether PO activities are enhanced or reduced by fertilizer application.

Soil samples were taken from plots in a 14-year-old experimental walnut orchard, subjected, since planting, to three fertilizer treatments: organic (compost) or mineral (urea) fertilization at the same rate of nitrogen application (100 kg N ha^{-1}), or left unfertilized. Soil samples were divided according to sampling depth (0–20 cm and 20–40 cm).

Results showed that the application of compost significantly increased C and N pools. qCO_2 and DHD/C_{ext} data indicated that the biota at 20–40 cm soil depth was more stressed or mainly composed of microorganisms with low substrate utilization efficiency. Phenol oxidase, tyrosinase, and catechol oxidase activities were significantly promoted in the surface layer by compost addition. In contrast, laccase activity showed a large increase in the deeper soil layer when supplied with mineral N, whereas compost addition led to increased activity in the surface layer. These findings suggest that soil phenol oxidases play a part in the determination of soil functionality, but they need to be investigated in greater depth in order to understand the mechanisms regulating their activities.

1. Introduction

Soil organic carbon (SOC) content is considered an essential factor in determining soil quality. Its decline can be counteracted and prevented effectively through the adoption of appropriate agricultural management practices (Lal, 2004). One such widespread agronomic practice is nitrogen (N) application. It affects microbial community composition and metabolic activity and can have long-term effects on the SOC cycle (Carreiro et al., 2000). Moreover, given the interconnection between soil N and carbon (C), external addition of N can also influence cellular C:N stoichiometric ratios and soil ecological stoichiometry, thus leading to changes in C use efficiency (Finn et al., 2016).

Microbes react to changes in substrate availability (i.e. C or N excess/limitation) by increasing or decreasing their respiration rate, and using C to gain energy or to increase biomass (Spohn, 2015). Moorhead and Sinsabaugh (2006) proposed a classification of microorganisms involved in the decomposition of different litter pools: *opportunists* that are active in the early stage of decomposition, using the available litter directly; *decomposers* that degrade cellulose and lignocellulose by expressing both hydrolytic and oxidative enzymes; and *miners* that degrade humified organic matter by using oxidative enzymes (such as phenol oxidase) that are able to metabolize phenols and degrade the most recalcitrant pools. Therefore, microorganisms may regulate enzyme expression depending on decomposition stage or characteristics of the substrate present in the soil. Furthermore, soil enzymes may show considerable changes in activity and interaction with soil components, leading to changes in SOC turnover after disturbance due to management practices (Naseby and Lynch, 2002). It should be highlighted that enzyme activities have the capacity to adapt to soil perturbations swiftly, and therefore they can be considered timely indicators of soil quality also in the case of small-scale disturbances (Cattaneo et al.,

* Corresponding author at: Viale Fanin 40, 40127 Bologna, (Italy).
E-mail address: luciano.cavani@unibo.it (L. Cavani).

https://doi.org/10.1016/j.geoderma.2017.12.009
Received 14 June 2017; Received in revised form 4 December 2017; Accepted 8 December 2017
Available online 18 December 2017

2014). In addition, it is important to underline that enzyme activity is controlled by not only availability and characteristics of soil substrates but also by abiotic conditions such as pH, temperature, presence of heavy metals, and soil moisture. These abiotic conditions have an impact on microbial biomass, affecting enzyme production, kinetics, stability, and affinity for substrates (Rao et al., 2014). In general, the main reason for measuring hydrolytic enzyme activity is the role the enzymes play in mediating soil biochemical processes related to nutrient cycles (Giacometti et al., 2014). However, these processes are also controlled by oxidative enzymes, which, although equally important in soil dynamics, are less well studied. In order to obtain a general picture of soil quality, we therefore need to investigate the activity not only of hydrolytic enzymes but also of oxidative enzymes.

One of the main, and most studied, groups of enzymes involved in oxidative processes is phenol oxidases (POs) (Stursova and Sinsabaugh, 2008), which are extracellular oxidoreductase enzymes. They are released into the environment through excretion or cellular lysis. Once in the soil, they can oxidize phenolic compounds and degrade lignin and humic substances, permitting the release of C and other nutrients (Piotrowska-Dlugosz, 2014; Sinsabaugh, 2010). Previous studies demonstrated that POs apparently behave differently from hydrolytic enzymes, and therefore, their activities are often not correlated (Stursova and Sinsabaugh, 2008). For example, it has been observed that, in contrast to PO activities, hydrolytic activities tend to decrease with increasing soil depth (Jackson et al., 2009). In addition, activities such as carbohydrate hydrolysis increase after N fertilization, whereas oxidative enzyme activities tend to decline (Moorhead and Sinsabaugh, 2006). This suggests that PO activities are controlled by factors different from those controlling hydrolytic activities, and some soil processes seem to be mediated mostly by oxidative enzymes. For instance, Carreiro et al. (2000) have observed that in ecosystems where litter is low in lignin and N content (i.e. grasslands), enrichment with N increases the rate of organic matter decomposition, whereas the rate decreases in litter with high lignin content (i.e. temperate and boreal forests). The rate of decomposition was correlated with PO activity; the correlation was positive in correspondence to higher organic matter decomposition rate, whereas it was negative with lower organic matter decomposition rate (Allison and Vitousek, 2004; Carreiro et al., 2000). Schimel and Weintraub (2003) studied a N-limited model in which adding inorganic N produced a reduction in the microbial respiration rate (determined with the metabolic quotient, qCO_2); this may be connected to the inhibition of ligninolytic enzymes that allow formation of more recalcitrant humic complexes.

Correlations between PO activities and hydrolytic enzyme activities, soil depth, and N application have been studied in ecosystems such as grasslands and forests. However, they are yet to be investigated in detail in agricultural systems where soil fertility is an important factor and organic matter content depends on agronomic management practices, such as fertilization, grassing, and crop rotation. We investigated the relationships between soil PO activities and the long-term effect of organic (compost) and mineral fertilization in an experimental walnut orchard. Fertilization practices can indeed have a great impact on soil fertility, not only through nutrient inputs entering soil, but also through organic matter decomposition rates (Giacometti et al., 2013). For example, both organic and inorganic N inputs induce a reduction in the degradation of recalcitrant C related to decreases in enzymes (such as PO) responsible for its decomposition (Craine et al., 2007).

In this context, we assessed changes in soil PO activities in an agricultural system, taking into account the effects of N application. Based on the results of previous studies in natural ecosystems (Allison and Vitousek, 2004; Carreiro et al., 2000; Jackson et al. 2009; Stursova and Sinsabaugh 2008), we expect to see differences between PO and hydrolytic microbial activities. In addition, we expected a reduction in PO activities as a consequence of N application that would bring greater stabilization of soil organic carbon estimated, for example, with the $\delta^{13}C$ measure (Balesdent and Mariotti, 1996; Ladyman and Harkness, 1980).

The goal of this study was, therefore, to evaluate: (i) whether trends in PO activities differed from those in microbial biomass and activity, and in particular, from those in hydrolytic enzymatic activities; and (ii) whether fertilization management influenced PO activities, and whether organic or mineral N fertilizer input enhanced or reduced PO activities.

2. Materials and methods

2.1. Study site and experimental design

The experimental site belongs to the University of Bologna and is located in Cadriano (BO) in the southern Italian Po Valley (45.53° N, 11.38° E, 28 m above sea level), which is an area characterized by high SOC depletion as a consequence of intensive agricultural exploitation (Malucelli et al., 2014). The experimental system was also chosen to enable a trial lasting for 14 years, a sufficiently long period to have a consolidated agrosystem where responses are not the result of temporary perturbations. Mean annual precipitation and temperature for the area are 747 mm and 14.2 °C respectively. A walnut (*Juglans regia* L.) orchard, consisting of the cultivar 'Lara' grafted on to seedling rootstock, was established in 2001 in a 7 m × 8 m (179 trees ha^{-1}) grid on soil classified as fine-silty, mixed, superactive, mesic Udifluventic Haplustepts (USDA Soil Taxonomy), characterized by a basic sequence of horizons (Oi, A, Ag, Bg) and formed on alluvial sediment. The soil was initially characterized as sub-acid pH (6.8), with low content of active lime (< 30 g kg^{-1}), organic matter (11 g kg^{-1}), and total N (1 g g^{-1}), whereas the soil C/N ratio was 6.4.

The climate of the area is classified as temperate sub-continental with cold winters and humid and warm summers. From the second growing season onwards, the orchard floor was not tilled, and maintained permanently with a mix of grass species (*Lolium perenne* L., *Festuca rubra* L., *and Poa pratensis* L.) mown twice a year. Trees, managed in terms of pruning, irrigation, and pest and disease control according to the regional guidelines for integrated crop management, were planted out in five rows, each with 20 plants, for a total field length of 135 m and a width of 40 m.

From the time of planting, the orchard was divided into plots, each subjected to one of three different fertilizer treatments: i) unfertilized control; ii) with organic amendments; and iii) with mineral inputs. Organic and mineral treatments received N at a rate of 100 kg ha^{-1} $year^{-1}$, applied to the soil surface without tilling, while unfertilized control plots did not receive any external input. The organic N source was compost produced from a mix of organic municipal wastes, pruning material from urban ornamental trees and gardens, and agro-industrial organic residues, following a 3-month biological stabilization period under aerobic conditions (Table 1). Compost analysis revealed low presence of heavy metal (Cr (VI), Pb, Cu, Cd, Ni, Zn, and Hg) concentrations (Table 1). Compost was spread during the flowering period with rates of application adjusted annually. Commercial urea (46% N) was the N source applied to the mineral treatment plots, with total N rate split yearly between the flowering (60%) and postharvest (40%) periods. The treatments were compared using a randomized block design with five replicates, arranged in five adjacent rows of trees. Experimental units (replicates), each consisting of four consecutive trees, were randomly distributed within each row.

2.2. Soil sampling and storage

In May 2015, soil samples from the top 40 cm soil profile were collected from the middle of each replicate equidistant from the nearest trunks and inside the leaf canopy (one sample from every experimental unit up to a total of 15 soils samples). Rainfall during the 4–6 weeks preceding sampling was average and therefore, at the time of sampling, soils were relatively moist (15–20% of fresh weight). Each sample was