Lasting influence of biochemically contrasting organic inputs on abundance and community structure of total and proteolytic bacteria in tropical soils

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ARTICLE INFO

ABSTRACT

The SOM field experiments in Kenya, which have been initiated in 2002 on two contrasting soils (clayey Humic Nitisol (sand: 17%; silt: 18%; clay: 65%) at Embu, sandy Ferric Alisol (sand: 66%; silt: 11%; clay: 22%) at Machanga), were used for exploring the effect of nine year annual application of biochemically contrasting organic inputs (i.e., *Zea mays* (ZM; C/N ratio: 59; (lignin + polyphenols)-to-N ratio: 9.8); *Tithonia diversifolia* (TD; 13; 3.5); *Calliandra calothyrsus* (CC; 13; 6.7)) on the soil bacterial decomposer community. Soil samples were taken at the onset of the rainy season before application of fresh organic inputs in March 2011. We studied the abundance (quantitative PCR) and community structure (T-RFLP analysis) of the total (i.e., 16S rRNA gene) and specifically proteolytic (i.e., *npr* gene encoding neutral metalloproteases) bacteria. Alterations of the soil microbial decomposer community were related to differences of quantity (i.e., soil carbon (TC)) and particularly composition of SOC, where mid-infrared spectroscopic (DRIFTS) information, and contents of extractable soil polyphenol (PP) and the newly introduced PP-to-TC ratio served as SOC quality indicators. For total bacteria, effect of organic input quality was minor in comparison to the predominant influence of soil texture. Elevated soil PP content, driven by polypheneol rich organic inputs, was not suppressive for overall bacterial proliferation, unless additional decomposable C substrates were available as indicated by PP-to-TC ratios. In contrast to the total bacterial community, biochemical quality of organic inputs exposed a stronger effect on functionally specialized bacterial decomposers, i.e., proteolytic bacteria. The *npr* gene abundance was depressed in the TD treated soils as opposed to soils receiving CC, and showed a positive correlation with soil PP. It was suggested that the high presence of lignin and polyphenol relative to the N content in organic inputs was increasing the *npr* gene abundance to counteract most likely the existence of polyphenol−protein complexes aggravating protein degradation. We concluded from our study that integration of spectroscopic, geochemical (i.e., soil PP) and molecular soil data provides a novel pathway to enhance our understanding of the lasting effect of organic input quality induced SOC quality changes on bacterial decomposers and particularly proteolytic bacteria driving soil organic N cycling.

1. Introduction

In tropical, small-holder agro-ecosystems, microbial decomposition and mineralization of organic inputs provide a critical means to sustain soil productivity (Vanlauwe et al., 2010). In particular, the biochemical quality of organic inputs, which is mainly characterized by their content of nitrogen (N), cellulose, lignin and particularly polyphenols (Wardle and Giller, 1996; Palm et al., 2001), has been shown to determine the synchrony of crop nutrient supply with actual crop demand (Balser and Firestone, 2005; Vanlauwe et al., 2010). The pivotal role of soil decomposing microorganisms on crop nutrient synchronization was already earlier postulated, but the actual regulatory effect of organic input quality on functionally relevant soil microorganisms in e.g. N cycling is, however, still poorly elucidated (Rasche and Cadisch, 2013).

Recent reports on soil microbial N cycling to date focused primarily on structural composition and abundance of functional genes responsible for prokaryotic nitrification and denitrification (e.g., Francis et al., 2005; Leininger et al., 2006; Hai et al., 2009). Comparably, the ecological significance of bacterial proteolysis (i.e., cleavage of amino acid bonds of organic input-derived proteins) has been so far mostly overlooked (Weintraub and Schimel, 2005; Vranova et al., 2013). In order to better understand N supply to crops through application of biochemically contrasting organic inputs in tropical, resource-limited agro-ecosystems, their effects on soil proteolytic microbial communities need to be, however, considered. This is justified as the transformation of organic input derived N (i.e., proteins) to mineral, plant available N is predominantly accomplished through bacterial proteolysis as the initial step in soil N cycling including nitrification (Rasche and Cadisch, 2013).

Most soil microorganisms express proteolytic activities by synthesizing an array of different proteases and peptidases (Vranova et al., 2013). Of these, neutral metalloproteases (Npr) were shown to encompass a fundamental role in protein degradation in many agricultural soils (Bach and Munch, 2000; Kamimura and Hayano, 2000; Sakurai et al., 2007). Accordingly, Bach et al. (2002) developed an oligonucleotide set targeting neutral metallopeptidase (npr gene) which was used to reveal differences in abundance, expression and phylogeny of proteolytic bacterial populations in response to soil management, soil type and season (Bach et al., 2002; Sakurai et al., 2007; Mrkonjic Fuka et al., 2008, 2009). Although the presence and activity of bacterial proteases in various tropical soils were confirmed (Insam et al., 1999; Wick et al., 2002; Oseni et al., 2007), there is only limited information available to which extent abundance and diversity of bacterial proteolytic genes in tropical agro-ecosystems are controlled by biochemically contrasting organic inputs. Recently, Sakurai et al. (2007) showed on basis of denaturing gradient gel electrophoresis analysis that, in comparison to inorganic fertilizer, organic inputs (i.e., farm yard manure, rice bran) altered significantly the composition of npr genes in arable soils.

It has been reported for tropical agro-ecosystems that organic inputs (e.g., *Tithonia diversifolia*) rich in organic N (>2.5%), but poor in polyphenols (<4%) are subjected to fast decomposition, thus releasing a considerable amount of N in the first weeks after application to soil (Chivenge et al., 2009; Gentile et al., 2009). In contrast, organic inputs, which contain, apart from high organic N (>2.5%), also high amounts of polyphenols (>4%) (e.g., *Calliandra calothyrsus*), release N gradually so that only a small amount of organic input derived N is actually available for the succeeding crop although it remains in soil (Chivenge et al., 2009; Gentile et al., 2009). It was earlier suggested that this delayed release of protein-derived N may be the consequence of the ability of plant polyphenols to bind proteins, thus protecting these against microbial degradation (Handayanto et al., 1997; Mutabaruka et al., 2007).

Consequently, the presence of organic input derived polyphenols in soils requires special attention when emphasizing the long-term effect of biochemically contrasting organic inputs on soil microbial N cycling including particularly bacterial proteolysis. The direct determination of polyphenol contents in soils via the commonly used Folin−Ciocalteu approach remains, however, difficult as the concentration of polyphenols in soils are, relative to total soil organic carbon (SOC), critically low (Suominen et al., 2003; Kanerva et al., 2008). Hence, the application of this detection method may be particularly disadvantageous for tropical soils as these are commonly highly weathered and characterized by fast proceeding microbial decomposition of organic inputs, whereby only a small proportion of applied organic matter (including polyphenols) is sequestered in the SOC pool (Fearnside, 2000; Jörgensen and Castillo, 2001; Oelbermann et al., 2004).

Alternatively, diffuse reflectance Fourier transform mid-infrared spectroscopy (DRIFTS) represents − compared to commonly applied physical and chemical SOC fractionation techniques (e.g., von Lützow et al., 2006) − an appropriate method to characterize the biochemical composition of SOC as altered by contrasting organic input types (e.g., Haberhauer et al., 2000; Antil et al., 2005; Gerzabek et al., 2006). DRIFTS detects vibrational bendings and stretchings of functional organic groups which are visualized in the mid-infrared spectrum ranging between 4000 and 400 cm^{-1}. DRIFTS profiles contain information of main functional groups of SOC reaching from labile (e.g., aliphatic) to recalcitrant (aromatic, phenolic) compounds (e.g., Baes and Bloom, 1989; Janik et al., 2007; Tatzber et al., 2010; Demyan et al., 2012; Duboc et al., 2012). DRIFTS has been recently used to detect functional groups (e.g., polyphenols at 1270 cm^{-1} (Janik et al., 2007)) of SOC by integrating respective peak areas of distinct spectral frequencies. Additionally, Demyan et al. (2012) used peak areas at 1620 cm^{-1} to characterize the effect of farm yard manure on aromatic compounds within the SOC pool, while Gerzabek et al. (2006) found peak heights at 2920, 1630 and 1450 cm^{-1} of soils to correlate with SOC contents of a Eutric Cambisol.

In the present study, it was our primary objective to explore if long-term application of biochemically contrasting organic inputs to two pedogenetically different tropical soils induced significant effects on the abundance and community structure of total and specifically proteolytic soil bacteria. We proposed that these alterations of total and proteolytic bacteria occurred due to organic input quality driven changes of the SOC composition considering specifically soil polyphenols as regulators of abundance and community structure of soil proteolytic bacteria harboring the npr gene.

2. Materials and methods

2.1. Field experiments and soil samplings

Soil samples were obtained from the SOM field experiments which were initiated in Kenya in March 2002 to determine primarily the influence of continuous annual application of organic inputs of different biochemical quality on SOC dynamics (Gentile et al., 2008). A detailed field experiment description can be retrieved from Chivenge et al. (2009). The study sites are located in the central highlands of Kenya, Embu ("E"; 0°30′ S, 37°27′ E; 1380 m above sea level (a.s.l.)) and Machanga ("M"; 0°47′ S, 37°40′ E; 1022 m a.s.l.). Mean annual rainfall is 1200 mm in Embu and 900 mm in Machanga, which occurs in two distinct rainy seasons within the course of the year. The mean annual temperature is 20 °C for Embu and 26 °C for Machanga. The soil at Embu is defined as a Humic Nitisol (sand: 17%; silt: 18%; clay: 65%), while a Ferric Alisol (sand: 66%; silt: 11%; clay: 22%) is characteristic for Machanga (FAO, 1998).

The soil treatments are similar at both experimental sites including various biochemically contrasting input types according to the definition by Palm et al. (2001) (Table 1). The following organic inputs were selected for the present study: *T. diversifolia* ("TD"; class I), *C. calothyrsus* ("CC"; class II), and *Zea mays* ("ZM"; class III). Biochemical quality of used organic inputs was described previously in Gentile et al. (2011) (Table 1). A control with no organic inputs (CON) was also included. Organic inputs (4 Mg C ha^{-1}) are annually incorporated into the soil prior to the start of the long rains starting in March.

Soil sampling was performed at the onset of the rainy season before application of fresh organic inputs in March 2011, nine years after experiment start. For the present study, we have selected only those plots without mineral N fertilizer to exclude the effect of