Soil Biology & Biochemistry 74 (2014) 204–213

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

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

Frank Rasche [a,*], Mary K. Musyoki [a], Carolin Röhl [a], Esther K. Muema [a], Bernard Vanlauwe [b], Georg Cadisch [a]

[a] Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany
[b] International Institute of Tropical Agriculture (IITA)-Kenya, P.O. Box 30709, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form
26 February 2014
Accepted 21 March 2014
Available online 2 April 2014

Keywords:
Organic input quality
SOC composition
Bacterial decomposers
Proteolytic genes
N cycling
Tropical soils

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).

* Corresponding author. Tel.: +49 (0) 711 459 24137; fax: +49 (0) 711 459 22304.
E-mail address: frank.rasche@uni-hohenheim.de (F. Rasche).

http://dx.doi.org/10.1016/j.soilbio.2014.03.017
0038-0717/

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

Table 1
Biochemical quality parameters[a] of the three studied organic inputs.

Input type	Biochemical quality class[b]	C [%]	N [%]	C-to-N ratio	ADF-lignin [%]	Polyphenols [%]	(Lignin + Polyphenols)-to-N ratio
Zea mays	III	40 (1)[c]	0.7 (0.1)	59 (11)	5.4 (1.2)	1.2 (0.2)	9.8 (2.2)
Calliandra calothyrsus	II	44 (0)	3.3 (0.2)	13 (2.6)	13.0 (2.6)	9.4 (1.6)	6.7 (0.6)
Tithonia diversifolia	I	38 (1)	3.2 (0.5)	13 (2)	8.9 (0.8)	1.7 (0.8)	3.5 (0.5)

[a] Data were taken from Gentile et al. (2011).
[b] Biochemical quality classes were defined by Palm et al. (2001).
[c] Standard deviation is given in parentheses.

mineral N on SOC and microbial community dynamics. From the three replicate plots of each treatment, ten soil cores from 0 to 15 cm were randomly taken using a soil auger with 3 cm diameter. Soil samples from each plot were bulked and transported to the laboratory in cooled boxes. Field fresh soil samples were made to pass through a 2 mm mesh, freeze-dried and shipped to University of Hohenheim for further analysis. There, the soil samples were stored under dry and dark conditions until laboratory analyses were started.

2.2. Microbiological soil analysis

2.2.1. Soil DNA isolation

Bulk soil DNA was isolated (FastDNA® Spin for Soil Kit, MP Biomedicals, Solon, Ohio, USA) and DNA extracts were quantified photometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

2.2.2. Microbial abundance

Prior to quantification of both target genes (i.e., 16S rRNA gene (total bacteria), *npr* gene (proteolytic bacteria)), amplicons from each investigated gene were generated for standard preparation. For 16S rRNA genes, PCR cocktails of 50 μl contained 1 ng DNA template isolated from *Escherichia coli* DSMZ 30083^T, 1× PCR reaction buffer (Bioline GmbH, Luckenwalde, Germany), 2.5 mM $MgCl_2$, 0.15 μM of each oligonucleotide (set Eub338::Eub518; Lane, 1991; Muyzer et al., 1993), 0.2 mM of each deoxynucleoside triphosphate (dNTP), and 2 U Biotaq™ DNA Polymerase (Bioline). PCR amplifications were performed under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles consisting of denaturation at 95 °C for 30 s, oligonucleotide annealing at 58 °C for 1 min, and polymerization for 2 min at 72 °C. Amplification was completed by a final extension at 72 °C for 7 min. For *npr* genes, PCR cocktails (50 μl) contained 20 ng DNA template isolated from *Bacillus cereus* DSMZ 310^T, 1× PCR reaction buffer (Bioline), 2 mM $MgCl_2$, 0.8 μM of each oligonucleotide (set nprI::nprII; Bach et al., 2002), 0.2 mM of each dNTP, 4 μg bovine serum albumin (Bioline) and 2.5 U Accuzyme *Taq* polymerase (Bioline). PCR amplifications were performed under the following conditions: initial denaturation at 94 °C for 5 min, 40 cycles consisting of denaturation at 94 °C for 30 s, oligonucleotide annealing at 53 °C for 30 s, and polymerization at 72 °C for 30 s. Amplification was completed by a final extension at 72 °C for 10 min. Amplicons were checked on 1% (w/v) agarose gels stained with GelRed™ (Biotrend Chemikalien GmbH, Cologne, Germany), purified (Invisorb Fragment CleanUp kit, Stratec Molecular GmbH, Berlin, Germany), ligated into the StrataClone™ PCR cloning vector pSC-A (Stratagene, La Jolla, CA, USA), and ligation products were transformed with StrataClone™ SoloPack® competent cells (Stratagene). Specificity of clones used as quantitative PCR (qPCR) standards were checked via sequencing at LGC Genomics GmbH (Berlin, Germany) and BLAST analysis. Plasmid DNA was isolated (GenElute™ Plasmid Miniprep Kit, Sigma–Aldrich) and quantified as described above.

For qPCRs of both target genes, 25 μl PCR cocktails were prepared containing 12.5 μl 1 × SYBR green master mix (16S rRNA gene: Power SYBR green master mix (Applied Biosystems, Foster City, CA, USA); *npr* gene: Brilliant III Ultra-Fast SYBR qPCR master mix (Agilent Technologies Inc., Santa Clara, CA, USA)), 0.4 μM of each oligonucleotide, 5 ng template DNA, and 0.25 μl of T4 gene 32 protein (500 μg ml^{-1}, MP Biomedicals). PCR reactions were run on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Reactions were started with 95 °C for 10 min to initialize polymerase activation, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 35 s and elongation at 72 °C for 45 s. Melting curve analysis of amplicons was conducted to confirm that fluorescence signals originated from specific amplicons and not from primer dimers or other artifacts. Each DNA sample was processed in triplicate reactions, while standard curves were generated using duplicate 10-fold dilutions of isolated plasmid DNA. Automated analysis of PCR amplicon quality and quantity was performed with StepOne™ software version 2.2 (Applied Biosystems).

2.2.3. Microbial community structure

Amplicons of 16S rRNA and *npr* genes were subjected to T-RFLP analysis according to Rasche et al. (2011). As prerequisite for T-RFLP analysis, all forward oligonucleotides were labeled with 6-carboxyfluorescein at the 5′ end. For 16S rRNA genes, PCR conditions and amplicon purification were similar to the protocol as described above. For *npr* genes, however, three replicate PCRs were generated according to the previously described protocol, pooled and concentrated according to Rasche et al. (2006a) to achieve a sufficient amplicon quantity for digestion. Concentrated amplicons were purified with the QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). For 16S rRNA genes, 200 ng of each amplicon were digested with 5 U *Alu*I (New England Biolabs (NEB) Inc., Ipswich, MA, USA) restriction endonuclease at 37 °C overnight. For *npr* genes, 200 ng of each amplicon were digested with 2 U *Alu*I (NEB), 2 U HypCH4V (NEB), and 2 U *Sac*II (NEB) restriction endonucleases at 37 °C overnight. Restriction enzymes were inactivated at 65 °C for 20 min. Prior to T-RFLP analysis, digests were purified (Sephadex™ G-50, GE Healthcare Biosciences, Waukesha, WI, USA) according to Rasche et al. (2006b) and 2 μl of each purified digest were mixed with 17.75 μl HiDi formamide (Applied Biosystems) and 0.25 μl internal size standard (500 ROX™ Size Standard, Applied Biosystems). Labeled terminal-restriction fragments (T-RFs) were denatured at 95 °C for 3 min, chilled on ice, and subsequently detected on an ABI 3130 Genetic Analyzer (Applied Biosystems). Peak Scanner software (version 1.0, Applied Biosystems) was used to compare relative lengths of T-RFs with the 500 ROX™ size standard and to compile electropherograms into numeric data sets in which fragment length and peak height greater than 50 fluorescence units were used for fingerprint comparison. T-RFLP profiles used for statistical analyses were normalized according to Dunbar et al. (2000).

2.3. Physico-chemical soil analysis

2.3.1. Mid-infrared spectroscopic analysis

Mid-infrared spectra of freeze-dried soil samples were recorded according to Rasche et al. (2013). Briefly, soil samples were ball-

milled and maintained overnight at 32 °C prior to analysis. Mid-infrared spectra of soil samples (250 mg) were recorded on a Tensor-27 Fourier transform spectrometer (Bruker Optik GmbH, Ettlingen, Germany) using a potassium bromide (KBr) beam splitter and a liquid N cooled mid-band mercury-cadmium-telluride detector. The Tensor-27 bench was mounted with a Praying Mantis diffuse reflectance chamber (Harrick Scientific Products, New York, USA) purged constantly with dry air derived from a compressor (Jun-Air International, Nørresundby, Denmark) with a flow rate of 200 l h^{-1}. Each soil sample was analyzed in triplicate from wavelengths 3950 to 650 cm^{-1} with 16 co-added scans per sample at a resolution of 4 cm^{-1} using solid, undiluted KBr (Carl Roth GmbH) as background. The acquisition mode was double forward-backwards and the Blackman–Harris-3 apodization function was used. The spectra were recorded in absorbance units (A.U.). By using the spectral processing software OPUS version 7.0 (Bruker Optik GmbH), obtained spectra were baseline corrected using the concave rubberband correction mode and the three laboratory repetitions of each soil sample were averaged.

For interpretation of whole DRIFTS fingerprints, A.U. at 1710 data points within the range from wavelength 3950 to 650 cm^{-1} of each recorded spectrum were used for statistical analysis (e.g., analysis of similarity, cluster analysis) as described below.

For particular DRIFTS band interpretation via peak area integration, it must be considered that both mineral and organic substances may have vibration frequencies in some of the same or overlapping wavenumbers (Demyan et al., 2012). Hence, after visual inspection of DRIFTS fingerprints (Fig. 1), only those frequencies were included which could be clearly related to polyphenolic compounds. According to a literature review, the following spectral frequencies were assigned as aromatic compounds and used for further statistical purposes: peak #1 (1750–1510 cm^{-1}) as defined as aromatic C=C and COO– stretchings (Smidt and Meissl, 2007; Nault et al., 2009; Demyan et al., 2012), and peak #2 (1450–1330 cm^{-1}) as defined as C–O of phenolic C–OH groups (Baes and Bloom, 1989). At these two identified DRIFTS peaks, upper and lower boundaries of wavelengths were established; a local baseline was drawn between the limits, and peak area integration was performed using the OPUS 7.0 software package to calculate the peak area (Demyan et al., 2012). DRIFTS peak areas were presented in the unit A.U.*cm^{-1}.

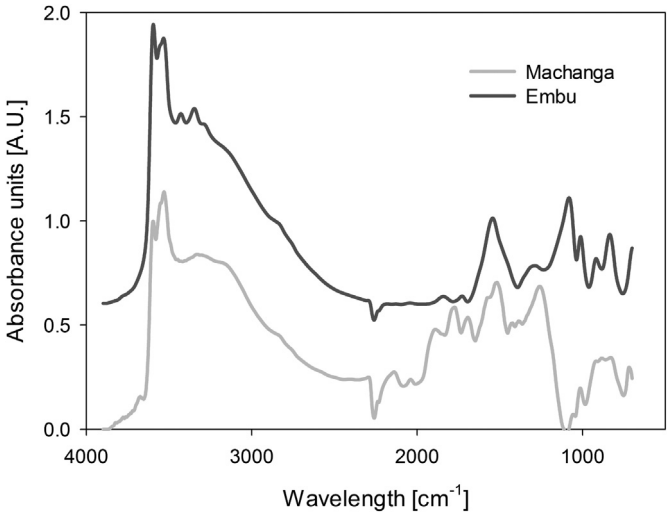

Fig. 1. Visualization of DRIFTS patterns obtained from the soils of the Embu and Machanga experimental field sites. The presented DRIFTS spectra are averaged spectra as calculated on basis of all four soil treatments ($n = 12$ as derived from plots CON, ZM, TD, and CC) as no significant differences were determined for the factor "Input type" (Table 2).

2.3.2. Total contents of soil polyphenols

For measurement of total soil polyphenol (PP) contents, freeze-dried soil samples were first ball-milled. Five gram of grounded soil were transferred into a 50 ml vessel, 10 ml of 70% aceton (Carl Roth GmbH, Karlsruhe, Germany) were added and mixed. The vessel was placed in an ultrasonic bath (model Elmasonic S30H; Elma Hans Schmidbauer GmbH, Singen, Germany) at 70 °C for 20 min. Every 5 min, the samples were mixed. Samples were centrifuged at 3000 rpm for 5 min, supernatant was transferred into a fresh 50 ml vessel and centrifugation was repeated. The supernatant was transferred into a 60 ml glass tube and placed in 70 °C heating block in which the liquid was evaporated completely under a permanent N_2-gas stream using a Techne Driblock DB-2D (Bibby Scientific Limited, Staffordshire, UK). The dry extract was supplemented with 3 ml 80% methanol (Carl Roth GmbH), mixed and incubated in the ultrasonic bath at 50 °C for 5 min for better resolving. The suspension was evaporated under the same conditions as described above until a final volume of 0.5 ml was reached. The concentrated sample was transferred into a 2.0 ml reaction vessel and centrifuged at 13,000 rpm for 5 min to pellet remaining suspended particles. The purified supernatant was transferred in a new 2.0 ml reaction vessel and mixed with 0.25 ml of a diluted Folin–Ciocalteu's phenol reagent solution (2:1 with distilled water; Sigma–Aldrich, St. Louis, MO, USA) and 1.25 ml of sodium carbonate solution (20%; Carl Roth GmbH). The 2 ml mixture was incubated in the dark at room temperature for 30 min. A calibration curve was prepared using tannic acid (Carl Roth GmbH) as reference substrate for polyphenols in known concentrations ranging from 0 to 100 mg ml^{-1}. Five hundred microliters of each reaction of both, soil and calibration samples, were measured in triplicates at 725 nm on a Specord 50 photometer (Analytik Jena AG, Jena, Germany). Total tannin (here used as total soil PP) concentration in soil samples was calculated against values obtained from the calibration curve and expressed in mg polyphenol kg^{-1} dry soil.

2.3.3. Total soil C and N contents

Total contents of total C and N in soil samples were determined by dry combustion according to DIN/ISO 13878 (1998) using a Vario-EL III Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

2.4. Statistical analysis

Normal distribution of physico-chemical and microbiological soil data was confirmed by non-parametric Kolmogorov–Smirnov tests (SPSS version 21 (SPSS 21) for Windows, IBM Corporation, Armonk, NY, USA). A two-factorial general linear model (SPSS 21) was used to determine significant treatment effects of the two factors "site" and "input type" on physico-chemical data and abundance of both investigated bacterial genes. Pearson's linear correlation coefficients were calculated for assessing significant relationships between gene abundance and physico-chemical soil parameters (SPSS 21).

Effects of the two factors "site" and "input type" on normalized T-RFLP and DRIFTS data sets were further assayed on basis of Bray–Curtis similarity coefficients (Legendre and Legendre, 1998). According to Rasche et al. (2011), a similarity matrix was generated for all possible pairs of samples of each target group. This similarity matrix was used for analysis of similarity (ANOSIM) statistics (Clarke and Green, 1988) to test if community structures of total and proteolytic bacteria as well as DRIFTS fingerprints were altered by the two factors. ANOSIM generates a test statistics, *R*. The

magnitude of R indicates the degree of separation between two independent communities, with a score of 1 indicating complete separation and 0 indicating no separation. For ANOSIM, a "global" R was first calculated to evaluate the overall effects of the two factors on the three individual data sets. In a second step, pairwise comparisons via ANOSIM were performed to reveal distinct effects of organic inputs on soil microbial communities and SOC composition at each study site (e.g., ECON (Embu-control) versus EZM (Embu-*Z. mays*)). In addition, Bray–Curtis similarity coefficients were used for cluster analyses using the "group average" clustering method considering the average values of the three field replications of each treatment. Calculation of similarity coefficients, ANOSIM and cluster analyses were carried out using Primer 6 for Windows (version 6.1.5, Primer-E Ltd., Plymouth, UK).

3. Results

3.1. Microbial community dynamics

Abundance of the *npr* gene was controlled significantly by the interaction of "site" and "input type" ($P < 0.05$) (Fig. 2A). Similar to the abundance of the 16S rRNA gene, gene copy numbers of the *npr* gene were lower in the TD treated Machanga soil as compared to the other treatments. Abundance of the *npr* gene showed acceptable positive correlations with N_t ($r = 0.463$), soil PP ($r = 0.458$) and (lignin + polyphenols)-to-N ratio ($r = 0.549$), while negative correlations were calculated when relating to the ratios between TC-to-N_t ($r = -0.441$) and PP-to-N_t ($r = -0.408$) ($P < 0.05$).

Abundance of 16S rRNA gene (total bacteria) was significantly affected by factor "site", where generally higher gene copy numbers were determined in the Embu soils ($P < 0.01$) (Fig. 2B). In contrast, copy numbers of the 16S rRNA gene did not show a significant response to factor "input type", although treatment TD tended to show lower gene copy numbers as compared to the other treatments at Machanga ($P > 0.05$). A positive correlation was calculated for the abundance of the 16S rRNA gene and *npr* gene ($r = 0.694$; $P < 0.01$). Furthermore, 16S rRNA gene copy numbers showed positive correlations with several physico-chemical soil parameters (TC: $r = 0.576$; $P < 0.01$; N_t: $r = 0.574$; $P < 0.01$; soil PP: $r = 0.504$; $P < 0.05$), while weak negative correlations were found with the PP-to-TC ratio ($r = -0.409$), TC-to-N_t ratio ($r = -0.409$), as well as peak #1 ($r = -0.435$) and peak #2 ($r = -0.452$) ($P < 0.05$).

Analysis of similarity (ANOSIM) of T-RFLP fingerprinting data revealed significant effects of factor "site" on the community structure of both genes (16S rRNA gene (total bacteria): $R = 0.361$ ($P < 0.01$); *npr* gene (proteolytic bacteria): $R = 0.318$ ($P < 0.05$)), while factor "input type" did not reveal any significant influence ($P > 0.05$) (Table 2). The distinct effects of factor "site" were further explored by cluster analyses based on group average clustering (Fig. 3A (*npr* gene) and 3B (16S rRNA gene)). Here, it became evident that the particular effects of the contrasting organic inputs on microbial communities in the soils of the Machanga site revealed greater community responses than compared to Embu. A proof for this trend was provided by pairwise ANOSIM, which calculated for 16S rRNA gene-based T-RFLP fingerprinting a distinct community difference between the two treatments MCON versus MTD ($R = 0.444$), while for *npr* gene T-RFLP analysis a remarkable community difference was determined between MCC versus MTD ($R = 1$).

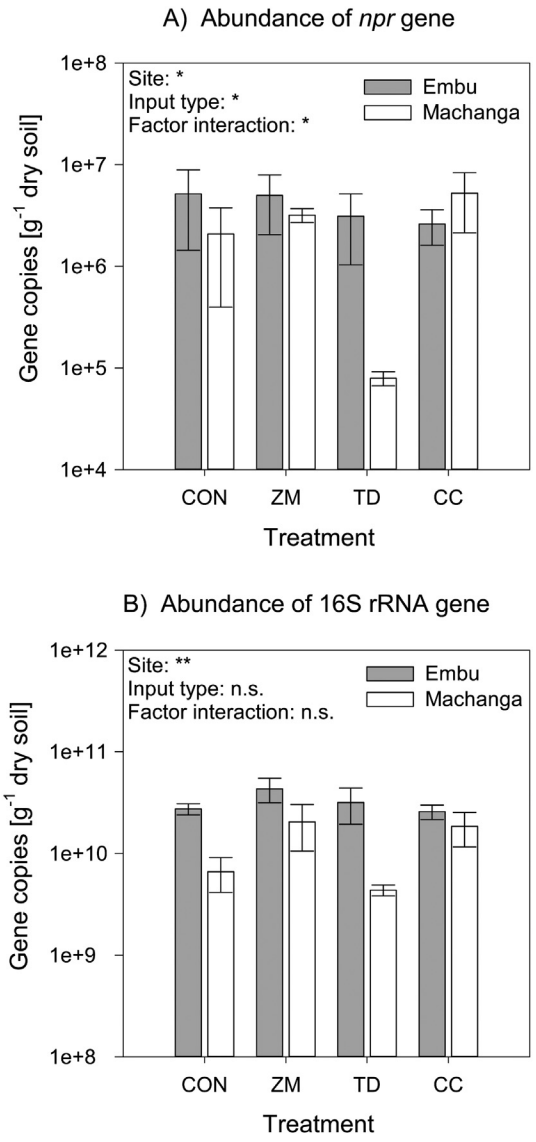

Fig. 2. Abundance of *npr* (A) and 16S rRNA (B) genes in the soils of the two experimental fields sites in Embu (gray columns) and Machanga (white columns). Presented data are average values calculated on basis of the three field replications along with standard deviation. Treatments are: CON = control with no organic inputs; ZM = *Zea mays*; TD = *Tithonia diversifolia*; CC = *Calliandra calothyrsus*.

Table 2
Global R values for the two main factors "site" and "input type" as obtained from the analysis of similarity of T-RFLP (16S rRNA and *npr* genes) and DRIFTS fingerprints.

Data set	Factor	
	Site	Input type
16S rRNA gene T-RFLP analysis	0.361**	n.s.
npr gene T-RFLP analysis	0.318*	n.s.
DRIFTS analysis	1.000***	n.s.

Significance levels: ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; n.s.: not significant ($P > 0.05$).

3.2. DRIFTS fingerprint interpretation

ANOSIM based on Bray–Curtis Similarity coefficients was used to interpret the DRIFTS spectra with respect to the effects of the two factors. A global R of 1 was calculated for factor "site" indicating a highly significant difference of the DRIFTS spectra between the soils of the two sites Embu and Machanga ($P < 0.001$) (Table 2). The differences were particularly visible in the spectral range from wavelengths 2200 to 650 cm^{-1} (Fig. 1). The following predominant peaks related to organic compounds occurred in the DRIFTS spectra

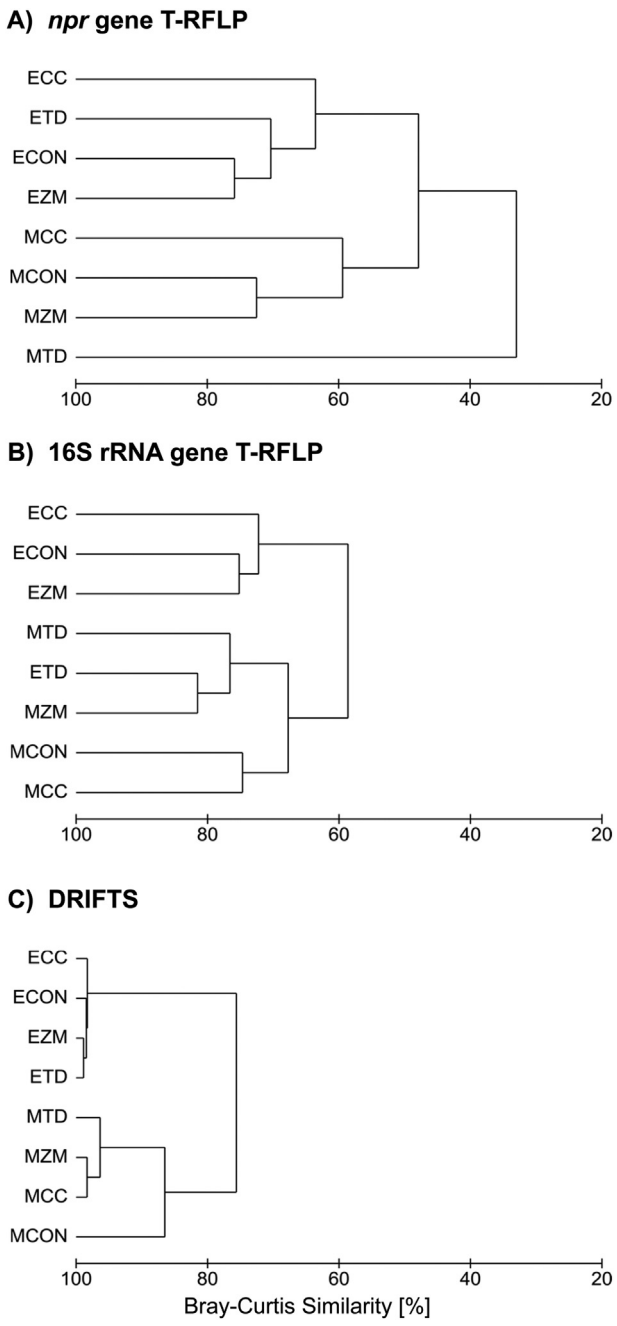

Fig. 3. Group average-based cluster analysis of T-RFLP (i.e., *npr* gene (A), 16S rRNA gene (B)) and DRIFTS (C) fingerprints as calculated on basis of the Bray–Curtis similarity coefficients. Treatments codes can be obtained from Table 3. Each treatment represents an average of $n = 3$ field replications.

obtained from both soils: carbohydrate overtones of C–OH stretching (2171–1932 cm^{-1}; Janik et al., 2007), aromatic C=C and COO– stretchings (1717–1558 cm^{-1}; Smidt and Meissl, 2007; Nault et al., 2009; Demyan et al., 2012), C=C of aromatic groups (1544–1500 cm^{-1}; Duboc et al., 2012), aromatic skeletal (1500–1458 cm^{-1}; Smidt and Meissl, 2007), C–O of phenolic C–OH groups (1450–1330 cm^{-1}; Baes and Bloom, 1989), C–OH of aliphatic OH (1194–1131 cm^{-1}; Tatzber et al., 2010), C–OH of aliphatic OH (1131–954 cm^{-1}) (Stevenson, 1994), and aromatic C–H out of plane bending (944–733 cm^{-1}; Baes and Bloom, 1989; Senesi et al., 2003).

These obvious differences of the DRIFTS fingerprints were further confirmed by group average-based cluster analysis (Fig. 3C). Contrastingly, factor "input type" did not reveal a significant global R for obtained DRIFTS spectra ($P > 0.05$) which was also confirmed by cluster analysis (Fig. 3C). However, direct comparison of differently organic input-treated soils at the Machanga site indicated a significant effect of organic input type on DRIFTS spectra (i.e., MCON versus MZM ($R = 0.778$), MCON versus MCC ($R = 0.852$), MCON versus MTD ($R = 0.778$)), while no significant organic input type effects were determined in the soils at Embu.

Peak area integration was performed for peak #1 (1750–1510 cm^{-1}), and peak #2 (1450–1330 cm^{-1}) as representatives for "aromatic" compounds of studied SOC pools. Both selected peak areas were affected by factor "site" ($P < 0.000$), but not by factor "input type" ($P > 0.05$) (Fig. 4). Generally, larger areas for both peaks were determined in the Machanga soils. Peak #1 revealed negative correlations with TC ($r = -0.967$; $P < 0.01$), N_t ($r = -0.876$; $P < 0.01$), and soil PP ($r = -0.583$; $P < 0.01$), while positive correlations were determined when relating to ratios between TC-to-N_t ($r = 0.600$; $P < 0.05$), PP-to-N_t ($r = 0.723$; $P < 0.01$) and particularly PP-to-TC ($r = 0.938$; $P < 0.01$). A similar trend was revealed for peak #2 ($r = -0.981$ (TC), $r = -0.900$ (N_t), $r = -0.577$ (soil PP), $r = 0.633$ (TC-to-N_t ratio); $r = 0.752$ (PP-to-N_t ratio), $r = 0.950$ (PP-to-TC ratio); $P < 0.05$). Both peaks did not show any correlation with biochemical quality parameters of the three organic input types ($P > 0.05$).

3.3. Chemical soil properties

The total contents of soil carbon (TC), soil nitrogen (N_t) and soil polyphenols (PP), TC-to-N_t ratio and PP-to-TC ratio were different between the two sites Embu and Machanga ($P < 0.01$), while factor "input type" did not expose any effect ($P > 0.05$) (Table 3). A significant interaction between the two factors was determined only for TC-to-N_t ratio ($P < 0.05$). There was a general trend that TC, N_t and soil PP showed higher values in the clayey Embu soils than found in the sandy Machanga soils (Table 3). In contrast, ratios of TC-to-N_t and PP-to-TC were generally lower in the Embu soils. Although the organic inputs did not change significantly the assayed chemical soil parameters, it was evident that those soils

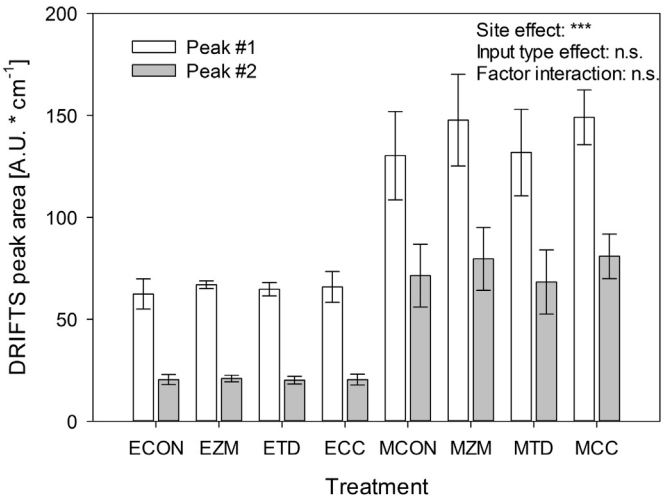

Fig. 4. Areas of the two studied peaks (peak #1 (1750–1510 cm^{-1}; aromatic C=C and COO– stretchings (Smidt and Meissl, 2007; Nault et al., 2009; Demyan et al., 2012)) and peak #2 (1450–1330 cm^{-1}; C–O of phenolic C–OH groups (Baes and Bloom, 1989))) as influenced by differently treated soil systems. Treatments codes can be obtained from Table 3. Each treatment represents an average of $n = 3$ along with standard deviation.

Table 3
Chemical characteristics of the differently treated soils at the experimental field sites in Embu and Machanga. A two-factorial general linear model was used to detect significant effects of the two factors "site" and "input type" as well as interactions between the two factors on studied chemical characteristics.

Site	Input type	Treatment code	Total soil C (TC) [%]	Total soil N (N_t) [%]	Total soil polyphenols (PP) [mg kg^{-1}]	TC-to-N_t ratio	PP-to-TC ratio	PP-to-N_t ratio
Embu	Control	ECON	2.17 (0.32)	0.26 (0.20)	27.9 (23.6)	9.4 (4.5)	12.5 (3.9)	109 (35)
	Zea mays	EZM	2.51 (0.54)	0.34 (0.13)	25.4 (16.2)	7.9 (4.4)	9.9 (2.0)	85 (37)
	Tithonia diversifolia	ETD	2.61 (0.32)	0.21 (0.05)	16.8 (5.8)	12.6 (2.7)	7.4 (0.6)	81 (15)
	Calliandra calothyrsus	ECC	2.24 (0.36)	0.20 (0.02)	16.5 (4.1)	11.4 (0.4)	6.4 (0.6)	87 (16)
Machanga	Control	MCON	0.41 (0.22)	0.02 (0.02)	11.6 (4.3)	20.4 (7.8)	29.6 (4.4)	728 (300)
	Zea mays	MZM	0.36 (0.21)	0.02 (0.02)	11.4 (6.2)	16.9 (4.8)	32.3 (1.1)	589 (178)
	Tithonia diversifolia	MTD	0.57 (0.37)	0.05 (0.03)	15.3 (4.2)	12.5 (3.1)	31.7 (2.4)	295 (37)
	Calliandra calothyrsus	MCC	0.38 (0.19)	0.03 (0.02)	11.9 (5.0)	14.5 (3.0)	29.0 (5.2)	628 (138)
Statistics								
"Site"			***	***	**	***	***	***
"Input type"			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction			n.s.	n.s.	n.s.	*	n.s.	n.s.

Standard deviation is given in parentheses.
Significance levels: ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; n.s.: not significant ($P > 0.05$).

treated with *T. diversifolia* (TD) tended to increase TC and N_t in the soils at both study sites. No clear trend was detected for the contents of soil PP and the PP-to-TC ratio. At Embu, soil PP was generally higher in the Control (CON) and *Z. mays* (ZM) plots as compared to TD and the *C. calothyrsus* (CC) plots. At Machanga, only the TD soils showed slightly higher soil PP contents than others at the same site.

TC displayed a positive correlation with soil PP ($r = 0.633$; $P < 0.01$), while a negative correlation was found between TC and PP-to-N_t ratio ($r = -0.716$; $P < 0.01$). TC and N_t revealed a negative correlation with the TC-to-N_t ratio ($r = -0.666$, $r = -0.829$; $P < 0.01$; respectively). Soil PP showed a positive correlation with N_t ($r = 0.637$; $P < 0.01$), but a negative correlation with the TC-to-N_t ratio ($r = -0.513$; $P < 0.05$). No correlations were obtained between chemical soil parameters and the biochemical quality of organic inputs ($P > 0.05$).

4. Discussion

4.1. N stress compensation of proteolytic bacteria

The major finding of the present study was – in contrast to the results obtained for the total bacterial community – the effect of organic input type on soil proteolytic bacteria which have been assayed on basis of the *npr* gene encoding neutral metalloproteases. Copy numbers of the *npr* gene were particularly depressed under the *T. diversifolia* (TD) treatment at Machanga, while they were significantly higher in the soils treated with *C. calothyrsus* (CC). Both organic input types had similar N contents, but differed greatly in the content of lignin and polyphenols. It has been acknowledged that high polyphenol and lignin contents reduce net residue N mineralization and hence availability of mineral N as a source for plant uptake and microbial metabolism (Fox et al., 1990; Constantinides and Fownes, 1994; Handayanto et al., 1997). Consequently, the regulative effect of lignin and polyphenols on the abundance of proteolytic bacteria was evidenced by the positive correlation between *npr* gene abundance and the residue (lignin+polyphenols)-to-N ratio substantiating the distinct response of the *npr* gene to limited N availability in the soil N pool (Fox et al., 1990; Constantinides and Fownes, 1994; Handayanto et al., 1997). Likewise, *npr* gene copy numbers revealed a positive correlation to soil PP. As PP form polyphenol–protein complexes, they further may reduce N availability and explain also the high *npr* gene abundance response of the other treatments (i.e., CON, ZM). Hence, both the presence of protein binding by polyphenols in soils as well as the high presence of lignin and polyphenol relative to N content of organic inputs of CC – expressed by a high PP-to-N_t ratio in contrast to TD – induced a critical N stress situation to which proteolytic bacteria responded with promoted synthesis of neutral metalloproteases to counteract the limited N availability (Melillo et al., 1982; Adamczyk et al., 2009; Triebwasser et al., 2012). This suggested that, although organic input derived N remained in the soil, proteolytic bacteria underwent a distinct promotion when soil PP was prevailing ("N stress compensation").

Similarly, a distinct shift towards fungal dominance was observed after long-term additions of polyphenol-rich residues of *Peltophorum dasyrachis* to soil resulting in stable polyphenol–protein complexes with reduced N availability (Mutabaruka et al., 2007). Accordingly, it could be assumed that such promotion of fungal communities might induce a stimulation of organic input dependent phenoloxidase activities as was recently shown by Kamolmanit et al. (2013). These authors reported that organic substrate-dependent N availability either promoted or inhibited phenoloxidase supporting the ongoing controversial discussion on how N actually determines fungal decomposition activities (e.g., Keeler et al., 2009; Edwards et al., 2011; Wu et al., 2011). Hence, it could be speculated for our study that fungal phenoloxidase activities, as promoted by sufficient N supply as energy source, might have considerably contributed to the lower PP contents in the TD soils compared to the other treatments.

The proposed "N stress compensation" concept was further evidenced by the fact that both DRIFTS peaks revealed a positive correlation of 16S rRNA gene abundance with the PP-to-N_t ratio. This indicated that microbial decomposition of recalcitrant C compounds including soil PP was, apart from the availability of decomposable C as critical energy source, essentially N-dependent. This explained the lower *npr* gene copy numbers observed in the Machanga soils compared to Embu. There, the low TC-to-N_t and high PP-to-N_t ratios in the TD treatment induced the low *npr* gene abundance as response to reduced N stress, e.g. higher relative N availability (Chivenge et al., 2011). It has to be considered that the presented study was based on soil samples which were obtained before application of fresh organic inputs, hence focusing clearly on longer term effects. Thus, it could be expected that the response to freshly applied inputs and availability of high protein amounts in TD might increase temporally the proteolytic activity in this treatment which may surpass other treatments including CC.

4.2. Minor influence of soil polyphenols on entire bacterial communities

The effect of long-term application of biochemically contrasting organic inputs on TC was more pronounced in the sandy Ferric

Alisol at Machanga as compared to the clayey Humic Nitisol at Embu; the latter having a much greater background SOC due to soil type specific (e.g., heavier texture) SOC stabilization mechanisms (Six et al., 2002; von Lützow et al., 2006; Chivenge et al., 2011). As confirmed by the positive correlation between TC content and 16S rRNA gene abundance, the higher TC content in the clayey Embu soils increased the bacterial abundance along with distinct alterations in the bacterial community structure since the start of the field experiment. It was shown earlier that soil type and specifically soil textural characteristics were critical determinants of the functional potential and community structure of soil decomposing microorganisms (Blackwood and Paul, 2003; Girvan et al., 2003; Rasche et al., 2006b). Interestingly, a positive correlation existed between 16S rRNA gene abundance and total soil PP. This indicated that extractable soil PP did not expose a suppressive effect on bacterial proliferation. On the other hand, the negative correlation between 16S rRNA gene abundance and PP-to-TC ratio suggested that soil PP did only have an indirect effect when there was a probable prevalence of additional, less recalcitrant C substrates available which derived from the native SOC pool and/or residual, non-decomposed organic inputs ("C resource compensation effect").

The essential interaction between bacterial abundance and TC as well as soil PP content further explained the negative correlations between 16S rRNA gene abundance and integrated areas of the two selected DRIFTS peaks (peak #1: 1750–1510 cm^{-1}, aromatic C=C and COO– stretchings; peak #2: 1450–1330 cm^{-1}, C–O of phenolic C–OH groups). Although a lower content of extractable soil PP were measured, greater DRIFTS aromatic peak areas – which presumably derived from the SOC pool – were calculated for the Machanga compared to the Embu soils. Additionally, both DRIFTS peaks showed a positive correlation with the PP-to-TC ratio. This substantiated again our assumption that overall availability of SOC relative to the presence of soil PP was regulative for proliferation of the total decomposing bacterial biomass.

Consequently, this difference in substrate availability and particular quality of SOC along with the more sandy soil texture at the Machanga site (Gentile et al., 2008) were obviously decisive for the lower bacterial abundance. It could be assumed that a critical proportion of applied organic inputs remained partially undecomposed and thus contributed most likely to the cPOM fraction (=coarse particulate organic matter > 250 μm) of the Machanga soils (Mafongoya et al., 1998; Palm et al., 2001). This assumption was supported by Samahadthai et al. (2010) and particularly Chivenge et al. (2011), where the latter report revealed an accumulation of organic input derived polyphenols in the light SOC fractions (i.e., cPOM) in the same soils explaining again the higher PP-to-TC ratios presented here. However, prospective research will have to clarify if organic input derived polyphenolic compounds in cPOM fractions – as regulatory determinants of abundance of microbial decomposers – contribute directly to the high increase of selected functional groups in the DRIFTS spectra obtained from the Machanga soils.

5. Conclusions and outlook

Long-term application of biochemically contrasting organic inputs exposed a stronger effect on functionally specialized bacterial decomposers, i.e., proteolytic bacteria, than on the total bacterial decomposer community. This effect was particularly evident in the sandy Machanga soils, but not in the clayey soils at Embu with its presumed stronger SOC background. Hence, it could be assumed that the interaction between soil textural characteristics with their distinct SOC stabilization mechanisms and biochemical quality of organic inputs were decisive for the recorded community alterations of decomposing and particularly proteolytic bacteria. Indications were provided that medium-term accessibility of SOC to the soil microbial decomposers depended on its location and stabilization within the mineral soil matrix. Conversely, we concluded that the abundance of decomposing bacteria (i.e., *npr* gene abundance) was obviously regulated by organic input quality. This had a distinct effect on N availability, which in turn might have contributed to the quantity (stabilization) and quality of SOC (Puttaso et al., 2013).

In the presented paper, the underlying mechanisms for these complex interactions between soil texture determining the SOC background, input quality and microbial decomposer (i.e., proteolytic bacteria) community remained however partially not fully understood. Hence, we propose on basis of the presented report that the integration of spectroscopic (as fast and reliable alternative to commonly used SOC fractionation procedures (e.g., von Lützow et al., 2006)) along with geochemical and molecular data can enhance the insight into organic input quality induced SOC quality changes on bacterial decomposers including particularly proteolytic bacteria. In this context, future research should also consider soil PP oxidizing soil fungi (Sinsabaugh et al., 2005; Edwards et al., 2011; Kamolmanit et al., 2013), which have not been addressed in this study, to get a closer insight into the underestimated dynamics of organic input type dependent soil PP contents.

The presented study was based on only two contrasting soils and a small variety of different organic inputs. It did not consider the acknowledged effects of e.g., soil management, seasonal alternations including accelerating climate variability, as well as species and growth stage of cultivated crops on the dynamics of microbial decomposition processes. Such influence factors need particular attention to get a better insight into the short- and long-term dynamics of soil proteolytic bacteria (Vranova et al., 2013). In this regard, it needs to be pointed out that the *npr* gene abundance data shown in this paper represented only the functional potential of neutral metalloprotease in the studied soils. To get a closer insight into the dynamics of active proteolytic bacteria, as regulated by biochemically contrasting organic inputs, we suggest the application of nucleic acid-based stable isotope probing approaches. This technique has approved its potential to demarcate those bacterial community members which were actively involved in the decomposition of contrasting organic inputs (España et al., 2011). Finally, prospective research should emphasize, apart from the significance of active *npr* genes in response to organic inputs, also other proteolytic genes (e.g., *sub* gene (encoding serine peptidase), *apr* gene (alkaline metallopeptidase), *pepN* gene (encoding alanine aminopetidase)) to substantiate the critical contribution of proteolytic bacteria to N mineralization and synchronized crop nutrient supply in agriculturally managed soils (Bach et al., 2002; Vanlauwe et al., 2010; Enowashu et al., 2012).

Acknowledgments

The authors thank Evonne Oyugi (Tropical Soil Biology and Fertility Institute of the International Centre for Tropical Agriculture (TSBF-CIAT), Nairobi, Kenya) for providing the soil samples from the SOM field experiments in Kenya. The authors thank Lena Rathjen and Elvise Mangoua (Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim) for their laboratory assistance. The first author (F. Rasche) is particularly grateful to the Life Science Center of the University of Hohenheim for the financial support within the program "Project support for young researchers". The contribution of Mary K. Musyoki was partially funded through the PhD program "Global Food Security" of the Food Security Center of the University of Hohenheim. The authors are further grateful to Professor Dr.

Ludwig Hölzle (Institute of Environmental and Animal Hygiene and Veterinary Medicine, University of Hohenheim) for access to his ABI 3130 Genetic Analyzer.

References

Adamczyk, B., Kitunen, V., Smolander, A., 2009. Polyphenol oxidase, tannase and proteolytic activity in relation to tannin concentration in the soil organic horizon under silver birch and Norway spruce. Soil Biology and Biochemistry 41, 2084–2093.

Antil, R.S., Gerzabek, M.H., Haberhauer, G., Eder, G., 2005. Long-term effects of cropped vs. fallow and fertilizer treatments on soil organic matter – I. Organic matter. Journal of Plant Nutrition and Soil Science 168, 108–116.

Bach, H.-J., Munch, J.C., 2000. Identification of bacterial sources of soil peptidases. Biology and Fertility of Soils 31, 219–224.

Bach, H.-J., Tomanova, J., Schloter, M., Munch, J.C., 2002. Enumeration of total bacteria with genes for proteolytic activity in pure cultures and in environmental samples by quantitative PCR mediated amplification. Journal of Microbiological Methods 49, 235–245.

Baes, A.U., Bloom, P.R., 1989. Diffuse reflectance and transmission Fourier transform infrared (DRIFT) spectroscopy of humic and fulvic acids. Soil Science Society of America Journal 53, 695–700.

Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395–415.

Blackwood, C.B., Paul, E.A., 2003. Eubacterial community structure and population size within the soil light fraction, rhizosphere, and heavy fraction of several agricultural systems. Soil Biology and Biochemistry 35, 1245–1255.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Comparison of organic versus mineral resource effects on short-term aggregate carbon and nitrogen dynamics in a sandy soil versus a fine textured soil. Agriculture, Ecosystems and Environment 140, 361–371.

Chivenge, P., Vanlauwe, B., Gentile, R., Wangechi, H., Mugendi, D., van Kessel, C., Six, J., 2009. Organic and mineral input management to enhance crop productivity in Central Kenya. Agronomy Journal 101, 1266–1275.

Clarke, K.R., Green, R.H., 1988. Statistical design and analysis for a 'biological effects' study. Marine Ecology Progress Series 46, 213–226.

Constantinides, M., Fownes, J.H., 1994. Nitrogen mineralization from leaves and litter of tropical plants: relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biology and Biochemistry 26, 49–55.

Demyan, M.S., Rasche, F., Schulz, E., Breulmann, M., Müller, T., Cadisch, G., 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. European Journal of Soil Science 63, 189–199.

DIN-ISO 13878, 1998. Bodenbeschaffenheit – Bestimmung des Gesamt-Stickstoffs durch trockene Verbrennung (Elementaranalyse). Beuth Verlag GmbH, Berlin, Wien, Zürich.

Duboc, O., Zehetner, F., Djukic, I., Tatzber, M., Berger, T.W., Gerzabek, M.H., 2012. Decomposition of European beech and black pine foliar litter along an alpine elevation gradient: mass loss and molecular characteristics. Geoderma 189–190, 522–531.

Dunbar, J., Ticknor, L.O., Kuske, C.R., 2000. Assessment of microbial diversity in four Southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Applied and Environmental Microbiology 66, 2943–2950.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., Pregitzer, K.S., 2011. Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. PLoS One 6 (6), e20421. http://dx.doi.org/10.1371/journal.pone.0020421.

Enowashu, E., Kandeler, E., Schloter, M., Rasche, F., Engel, M., 2012. Development of a primer system to study abundance and diversity of the gene coding for alanine aminopeptidase *pepN* gene in Gram-negative soil bacteria. Journal of Microbiological Methods 91, 14–21.

España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G.D., Cadisch, G., 2011. Identification of active bacteria involved in decomposition of complex maize and soybean residues in a tropical Vertisol using ^{15}N-DNA stable isotope probing. Pedobiologia 54, 187–193.

FAO, 1998. World Reference Base for Soil Resources. FAO, Rome, Italy.

Fearnside, P.M., 2000. Global warming and tropical land-use change: greenhouse gas emissions from biomass burning, decomposition and soils in forest conversion, shifting cultivation and secondary vegetation. Climate Change 46, 115–158.

Fox, R.H., Myers, R.J.K., Vallis, I., 1990. The nitrogen mineralization rate of legume residues in soil as influenced by their polyphenol, lignin, and nitrogen contents. Plant and Soil 129, 251–259.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences USA 102, 14683–14688.

Gentile, R., Vanlauwe, B., Six, J., 2011. Litter quality impacts short- but not long-term carbon dynamics in soil aggregate fractions. Ecological Applications 21, 695–703.

Gentile, R., Vanlauwe, B., van Kessel, C., Six, J., 2009. Managing N availability and losses by combining fertilizer-N with different quality residues in Kenya. Agriculture, Ecosystems and Environment 131, 308–314.

Gentile, R., Vanlauwe, B., Chivenge, C., Six, J., 2008. Interactive effects from combining fertilizer and organic residue inputs on nitrogen transformations. Soil Biology and Biochemistry 40, 2375–2384.

Gerzabek, M.H., Antil, R.S., Kögel-Knabner, I., Knicker, H., Kirchmann, H., Haberhauer, G., 2006. How are soil use and management reflected by soil organic matter characteristics: a spectroscopic approach. European Journal of Soil Science 57, 485–494.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied and Environmental Microbiology 69, 1800–1809.

Haberhauer, G., Feigl, B., Gerzabek, M.H., Cerri, C., 2000. FT-IR spectroscopy of organic matter in tropical soils: changes induced through deforestation. Applied Spectroscopy 54, 221–224.

Hai, B., Diallo, N.H., Sall, S., Haesle, r F., Schauss, K., Bonzi, M., Assigbetse, K., Chotte, J.-L., Munch, J.C., Schloter, M., 2009. Quantification of key genes steering the microbial nitrogen cycle in the rhizosphere of sorghum cultivars in tropical agroecosystems. Applied and Environmental Microbiology 75, 4993–5000.

Handayanto, E., Giller, K.R., Cadisch, G., 1997. Regulating N release from legume tree prunings by mixing residues of different quality. Soil Biology and Biochemistry 29, 1417–1426.

Insam, H., Bååth, E., Berreck, M., Frostegård, Å., Gerzabek, M.H., Kraft, A., Schinner, F., Schweiger, P., Tschuggnall, G., 1999. Responses of the soil microbiota to elevated CO_2 in an artificial tropical ecosystem. Journal of Microbiological Methods 36, 45–54.

Janik, L.J., Skjemstad, J.O., Shepherd, K.D., Spouncer, L.R., 2007. The prediction of soil carbon fractions using mid-infrared-partial least square analysis. Australian Journal of Soil Research 45, 73–81.

Jörgensen, R.G., Castillo, X., 2001. Interrelationships between microbial and soil properties in young volcanic ash soils of Nicaragua. Soil Biology and Biochemistry 33, 1581–1589.

Kamimura, Y., Hayano, K., 2000. Properties of protease extracted from tea-field soil. Biology and Fertility of Soils 30, 351–355.

Kamolmanit, B., Vityakon, P., Kaewpradit, W., Cadisch, G., Rasche, F., 2013. Soil fungal communities and enzyme activities in a sandy, highly weathered tropical soil treated with biochemically contrasting organic inputs. Biology and Fertility of Soils 49, 905–917.

Kanerva, S., Kitunen, V., Loponen, J., Smolander, A., 2008. Phenolic compounds and terpenes in soil organic horizon layers under silver birch, Norway spruce and Scots pine. Biology and Fertility of Soils 44, 547–556.

Keeler, B.L., Hobbie, S.E., Kellog, L.E., 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems 12, 1–15.

Lane, D., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, A., Goodfellow, M (Eds.), Nucleic Acid Techniques Systematics. John Wiley, West Sussex, UK, pp. 115–175.

Legendre, P., Legendre, L., 1998. Numerical Ecology, second English ed. Elsvier, Amsterdam.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809.

Mafongoya, P.L., Giller, K., Palm, C.A., 1998. Decomposition and nitrogen release patterns of tree prunings and litter. Agroforestry Systems 38, 77–97.

Melillo, J.M., Aber, J.D., Muratore, J.F., 1982. Nitrogen and lignin control hardwood litter decomposition dynamics. Ecology 63, 621–626.

Mrkonjic Fuka, M., Engel, M., Hagn, A., Munch, J.C., Sommer, M., Schloter, M., 2009. Changes of diversity pattern of proteolytic bacteria over time and space in an agricultural soil. Microbial Ecology 57, 391–401.

Mrkonjic Fuka, M., Engel, M., Gattinger, A., Bausenwein, U., Sommer, M., Munch, J.C., Schloter, M., 2008. Factors influencing variability of proteolytic genes and activities in arable soils. Soil Biology and Biochemistry 40, 1646–1653.

Mutabaruka, R., Hairiah, K., Cadisch, G., 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories. Soil Biology and Biochemistry 39, 1479–1492.

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.

Nault, J.R., Preston, C.M., Trofymow, J.A., Fyles, J., Kozak, L.M., Siltanen, R.M., Titus, B.D., 2009. Applicability of diffuse reflectance Fourier transform infrared spectroscopy to the chemical analysis of decomposing foliar litter in Canadian forests. Soil Science 174, 130–142.

Oelbermann, M., Voroney, R.P., Gordon, A.M., 2004. Carbon sequestration in tropical and temperate agroforestry systems: a review with examples from Costa Rica and southern Canada. Agriculture, Ecosystems and Environment 104, 359–377.

Oseni, O.A., Ekperigin, M.M., Akindahunsi, A.A., Oboh, G., 2007. Studies of physiochemical and microbial properties of soils from rainforest and plantation in Ondo state, Nigeria. African Journal of Agricultural Research 2, 605–609.

Palm, C.A., Gachengo, C., Delve, R., Cadisch, G., Giller, K.E., 2001. Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. Agriculture, Ecosystems and Environment 83, 27–42.

Puttaso, A., Vityakon, P., Rasche, F., Saenjan, P., Trelo-ges, V., Cadisch, G., 2013. Does organic residue quality influence carbon stabilization in tropical sandy soil? Soil Science Society of America Journal 77, 1001–1011.

Rasche, F., Trondl, R., Naglreiter, C., Reichenauer, T., Sessitsch, A., 2006a. Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum annuum* L.). Canadian Journal of Microbiology 52, 1036–1045.

Rasche, F., Hödl, V., Poll, C., Kandeler, E., Gerzabek, M.H., van Elsas, J.D., Sessitsch, A., 2006b. Rhizosphere bacteria affected by transgenic potatoes with antibacterial activities compared with the effects of soil, wild-type potatoes, vegetation stage and pathogen exposure. FEMS Microbiology Ecology 56, 219–235.
Rasche, F., Cadisch, G., 2013. The molecular microbial perspective of organic matter turnover and nutrient cycling in tropical agro-ecosystems – what do we know? Biology and Fertility of Soils 49, 251–262.
Rasche, F., Knapp, D., Kaiser, C., Koranda, M., Kitzler, B., Zechmeister-Boltenstern, S., Richter, A., Sessitsch, A., 2011. Seasonality and resource availability control bacterial and archaeal communities in soils of a temperate beech forest. ISME J. 5, 389–402.
Rasche, F., Marhan, S., Berner, D., Keil, D., Kandeler, E., Cadisch, G., 2013. midDRIFTS-based partial least square regression analysis allows predicting microbial biomass, enzyme activities and 16S rRNA gene abundance in soils of temperate grasslands. Soil Biology and Biochemistry 57, 504–512.
Sakurai, M., Suzuki, K., Onodera, M., Shinano, T., Osaki, M., 2007. Analysis of bacterial communities in soil by PCR–DGGE targeting protease genes. Soil Biology and Biochemistry 39, 2777–2784.
Samahadthai, P., Vityakon, P., Saenjan, P., 2010. Effects of different quality plant residues on soil carbon accumulation and aggregation formation in a tropical sandy soil in Northeast Thailand as revealed by a 10-year field experiment. Land Degradation and Development 21, 463–473.
Senesi, N., D'Orazio, V., Ricca, G., 2003. Humic acids in the first generation of EUROSOILS. Geoderma 116, 325–344.
Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M., Zak, D.R., 2005. Extracellular enzyme activities and soil carbon dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201–215.
Six, J., Feller, C., Denef, K., Ogle, S.M., Sa, J.C.D., Albrecht, A., 2002. Soil organic matter, biota and aggregation in temperate and tropical soils – effects of no tillage. Agronomie 22, 755–775.
Smidt, E., Meissl, K., 2007. The applicability of Fourier transform infrared (FT-IR) spectroscopy in waste management. Waste Management 27, 268–276.
Stevenson, F.J., 1994. Humus Chemistry. Genesis, Composition, Reactions, second ed. Wiley, New York. 496 pp.
Suominen, K., Kitunen, Smolander, A., 2003. Characteristics of dissolved organic matter and phenolic compounds in forest soils under silver birch (*Betula pendula*), Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). European Journal of Soil Science 54, 287–293.
Tatzber, M., Mutsch, F., Mentler, A., Leitgeb, E., Englisch, M., Gerzabek, M.H., 2010. Determination of organic and inorganic carbon in forest soil samples by mid-infrared spectroscopy and partial least squares regression. Applied Spectroscopy 64, 1167–1175.
Triebwasser, D.J., Tharayil, N., Preston, C.M., Gerard, P.D., 2012. The susceptibility of soil enzymes to inhibition by leaf litter tannins is dependent on the tannin chemistry, enzyme class and vegetation history. New Phytologist 196, 1122–1132.
Vanlauwe, B., Bationo, A., Chianu, J., Giller, K.E., Merckx, R., Mokwunye, U., Ohiopehai, O., Pypers, P., Tabo, R., Shepherd, K.D., Smaling, E.M.A., Woomer, P.L., Sanginga, N., 2010. Integrated soil fertility management: operational definition and consequences for implementation and dissemination. Outlook on Agriculture 39, 17–24.
von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B., Flessa, H., 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science 57, 426–445.
Vranova, V., Rejsek, K., Formanek, P., 2013. Proteolytic activity in soil: a review. Applied Soil Ecology 70, 23–32.
Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. Soil Biology and Biochemistry 28, 1549–1554.
Weintraub, M.N., Schimel, J., 2005. Seasonal protein dynamics in Alaskan artic tundra soils. Soil Biology and Biochemistry 37, 469–475.
Wick, B., Kühne, R.F., Vielhauer, K., Vlek, P.L.G., 2002. Temporal variability of selected soil microbiological and biochemical indicators under different soil quality conditions in south-western Nigeria. Biology and Fertility of Soils 35, 155–167.
Wu, L., Feinstein, L.M., Valverde-Barrantes, O., Kershner, M.W., Leff, L.G., Blackwood, C.B., 2011. Placing the effects of leaf litter diversity on saprophytic microorganisms in the context of leaf type and habitat. Microbial Ecology 61, 399–409.