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ORIGINAL PAPER

Soil fungal communities and enzyme activities in a sandy, highly weathered tropical soil treated with biochemically contrasting organic inputs

Bhanudacha Kamolmanit · Patma Vityakon · Wanwipa Kaewpradit · Georg Cadisch · Frank Rasche

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Abstract The regulative effect of long-term application of biochemically contrasting organic inputs such as rice straw (4.7 gN; 6.5 g polyphenols), groundnut stover (22.8 gN; 12.9 g polyphenols) and leaf litter of tamarind (13.6 gN; 31.5 g polyphenols) and dipterocarp (5.7 gN; 64.9 g polyphenols) on fungal decomposers was studied in a tropical sandy soil. Fungal decomposers were assayed by 18S rRNA gene-based community profiling and were combined with measurements of selected enzyme activities. Dipterocarp residue application depressed fungal abundance, but promoted specialized decomposers (e.g., *Aspergillus fumigatus* and *Anguillospora longissima*) with increases in polyphenol oxidase activity. The degree of functional redundancy for invertase and B-glucosidase activities was induced after the addition of easily decomposable rice straw and groundnut stover. Higher N availability in the tamarind treatment increased, in contrast to low N rice straw, fungal abundance (i.e., *Fusarium oxysporum*, *Myceliopthora thermophila*, and *Aspergillus versicolor*) and promoted invertase and B-glucosidase activities, while peroxidase activity was depressed. In addition, N availability seemed to regulate not only decomposing soil fungi, but also the abundance of protozoan decomposers whose actual contribution to N turnover in soils is still poorly understood. Prospective research should thus consider apart from studying decomposing fungi also protozoa and bacteria to better understand the microbially mediated degradation of complex organic materials in soils.

Keywords Organic input quality · Decomposition · Fungal abundance and community structure · Enzyme activities · Soil organic matter

B. Kamolmanit · P. Vityakon (✉) · W. Kaewpradit
Department of Plant Science and Agricultural Resources, Khon Kaen University, Khon Kaen, Thailand
e-mail: patma@kku.ac.th

B. Kamolmanit · G. Cadisch · F. Rasche
Institute of Plant Production and Agroecology in the Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany

Introduction

In tropical soils, intensive agricultural management on highly weathered sandy soils generally leads to a decline of soil organic matter (SOM) causing a serious depletion of soil productivity (Vityakon 2007). Incorporation of locally available organic inputs into degraded soils has been acknowledged to reconstruct crucial soil properties associated with SOM accumulation (Bierke et al. 2008; Puttaso et al. 2011a), aggregate stability (Samahadthai et al. 2010), and release of plant available nutrients from organic inputs through microbial decomposition and mineralization processes (Hadas et al. 2004; Vityakon 2007; Vityakon et al. 2000).

Biochemical quality of organic inputs as determined by their content of N, cellulose, lignin and polyphenols regulates microbial decomposition and mineralization processes (Palm et al. 2001; Swift et al. 1979; Wardle and Giller 1996). In this sense, it has been reported that high quality organic inputs (N >2.5 %, lignin <15 %, polyphenol <4 %) enhanced microbial biomass (Aciego Pietri and Brookes 2009; Debosz et al. 1999), microbial resource use efficiency (Wardle et al. 1999), net N mineralization (Vityakon and Dangthaisong 2005; Vityakon et al. 2000), as well as activities of enzymes including invertase, xylanase, and cellulase (Luxhøi et al. 2002; Sajjad et al. 2002; Stemmer et al. 1999). On the other hand, Abro et al. (2011) and Hadas et al. (2004) showed that high quality organic inputs (e.g., rice straw, tobacco, and rape

residues) generally possess reduced C sequestration potential in soils due to enhanced microbial decomposition leading to significant C losses via carbon dioxide release. To overcome this limitation, application of organic inputs of intermediate quality (lignin and polyphenols ranging from approximately 5 % to 15 % and 2 % to 10 %, respectively) were considered as applicable to enhance SOM accumulation, while guaranteeing the release of plant available nutrients from microbial decomposition and mineralization (Palm et al. 2001; Puttaso et al. 2011a). For tropical highly weathered sandy soils, Puttaso et al. (2011a) suggested, according to the definition by Palm et al. (2001), the use of organic inputs rich in organic N, but low in cellulose as well as with moderate lignin and polyphenol contents such as tamarind (*Tamarindus indica*; lignin= 87.7 gkg^{-1}, polyphenols=31.5 gkg^{-1}, N=13.6 gkg^{-1}). The authors observed, on the basis of a long-term field experiment in Northeast Thailand, after 13 years continuous tamarind application, an increase (stabilization) in SOM and promotion of resource use efficiency by the soil microbial decomposer community as compared to additions of low quality inputs such as rice straw. However, the underlying response of the soil microbial community and its turnover of organic matter as regulated by the availability and decomposability of organic inputs is not yet fully understood (Rasche and Cadisch 2013). This is particularly evident for the known occurrence of short-term increase in enzyme activities related to SOM decomposition (priming) in soils with functional redundant (e.g., cellulose degradation) and specialized (e.g., polyphenol degradation) decomposition activities of the soil microbial community (Kuzyakov and Bol 2006; Nannipieri et al. 2003).

Soil fungi have been shown to be predominant microorganisms in tropical sandy soils (Six et al. 2002). This was ascribed to their ability to decompose both, easily decomposable (e.g., cellulose, hemicellulose) and recalcitrant (e.g., lignin, polyphenols) organic inputs as well as their contribution to formation and stabilization of aggregates through fungal filaments and mucilage (Caesar-TonThat and Cochran 2001; España et al. 2011; Řzáčová et al. 2007; Yuste et al. 2011). Therefore, with regard to SOM stabilization in tropical sandy soils, prospective studies have to consider the long-term effect of biochemically contrasting organic inputs on the structure and function of the fungal decomposer community as key regulators of SOM stabilization. On the basis of nucleic acid-based community fingerprinting, it was shown that the structure of soil fungal communities responded sensitively to the biochemical quality of organic inputs regulating their availability and utilizability (España et al. 2011; Kelly et al. 2010). In the long-term perspective, it was reported that continuous application of biochemically contrasting organic inputs drove changes in fungal community composition and microbial enzyme activities (Nicolardot et al. 2007; Sinsabaugh et al. 2005). In this sense, microbial enzyme activities responded sensitively to C and N availability in soils as regulated by organic input quality (Aciego Pietri and Brookes 2009; Bissett et al. 2011; Lucas et al. 2007). Roldán et al. (2005) and Moscatelli et al. (2012) reported B-glucosidase activities as having positive correlations with C and N availability in soils treated with biochemically contrasting organic inputs. In contrast, B-glucosidase activities were inhibited by high lignin content of organic inputs (Luxhøi et al. 2002). It was further controversially discussed if microbial enzyme activities responsible for the decomposition of stable organic compounds (e.g., polyphenol oxidase, peroxidase) were either depressed or promoted in the presence of high soil N (Carreiro et al. 2000; Saiya-Cork et al. 2002).

To disentangle the complex nature of how organic input quality regulates decomposing soil microbial communities and their metabolic capabilities in tropical soils, we used a long-term field experiment on a highly weathered sandy soil in Northeast Thailand in which effects of biochemically different organic inputs on SOM dynamics have been studied for 16 years. Our primary hypothesis was that long-term application of biochemically contrasting organic inputs resulted in distinct shifts of the fungal community structure and that these community changes could be related to activities of selected enzymes involved in decomposition of carbonaceous organic compounds. The first objective of this study was to investigate how 16 years continuous application of biochemically contrasting organic inputs into a highly weathered sandy soil has altered the abundance and community structure of fungal populations as well as selected microbial enzyme activities. The second objective was to elucidate if increases in enzyme activities occurred with functionally redundant and/or specialized responses of the soil fungal community to newly added organic biochemically contrasting inputs.

Materials and methods

Soil sampling

Soil samples were collected from a long-term field experiment at the research station of the Agriculture and Co-operatives of the Northeast at Tha Phra subdistrict, Khon Kaen province, Thailand (16°20′N; 102°49′E). The soil was characterized as a Khorat Sandy Loam (fine loamy siliceous isohyperthermic Typic (Oxyaquic) Kandiustults (Soil Survey Staff 2006). At the beginning of the experiment in 1995, the top layer of the studied soils showed a sandy texture (sand, 93.4 %; silt, 4.5 %; and clay, 2.1 %), a bulk density of 1.45 gcm^{-3}, a pH (H_2O) of 5.5, a cation exchange capacity of 3.53 $cmol_c\ kg^{-1}$, organic C of 0.21 %, total N of 0.02 %, Bray II phosphorus of 47.2 $mgkg^{-1}$,

and exchangeable potassium of 0.077 $cmol_c\ kg^{-1}$ (Vityakon et al. 2000). The long-term field experiment was established with the primary objective to test the potential of various organic, annually applied inputs (at 10 $Mgha^{-1}$ rate) of contrasting biochemical quality (groundnut stover [*Arachis hypogeae*], tamarind [*Tamarindus indica*], dipterocarp [*Dipterocarpus tuberculatus*], and rice straw [*Oryza sativa*]) (Table 1) to restore SOM in a highly weathered sandy soil. Further details of the experimental design can be found in the work of Vityakon et al. (2000).

For the present study, soil samples were obtained from organic input-treated soils (native soil: N-soil) and unamended control plots (control soil: C-soil) in September 2011, 5 months after the organic inputs had been soil incorporated and 16 years after the field experiment had started. Soils used were defined as follows: unamended control soil (C), N-soils treated with rice straw (NRS), groundnut (NGN), dipterocarp (NDP), and tamarind (NTM). Relevant chemical characteristics (i.e., pH (H_2O), exchangeable Ca ($cmol_c\ kg^{-1}$), total C (gkg^{-1}), total N (gkg^{-1}), and soil C/N ratio) of these soils are shown in Table 2. After 16 years of yearly continuous residue application, NTM has accumulated the highest C, followed by NGN and NDP while NRS had the lowest C among the residue treated soils. Puttaso et al. (2011a) also found similar results in year 13 of the same long-term field experiment. They attributed this to the different quality of the residues which affected microbial processes involving in their decomposition. In addition, the long-term continuous application of the residues has resulted in changes in soil pH which again reflected their different quality. The significantly highest pH in NTM was related to the highest exchangeable Ca content (0.97 $cmol_c\ kg^{-1}$) while NGN and NDP had intermediate Ca contents (0.56 and 0.42 $cmol_c\ kg^{-1}$) and NRS had the significantly lowest Ca content (0.34 $cmol_c\ kg^{-1}$) (R. Roongthong and P. Lawongsa, personal communications). In contrast, the controlled soil had significantly lower pH than the treatments receiving the residues. This was again associated with lower exchangeable Ca in the controlled soil (0.14 $cmol_c\ kg^{-1}$) than the latter soils (R. Roongthong and P. Lawongsa, personal communications). It is likely that leaching of exchangeable bases was prevalent under the control, low CEC soil. Puttaso et al. (2011b) found in year 13 soils that the lowest total C in the control soil led to the lowest CEC in this soil relative to the residue treatments. Total C and N, representing SOM accumulation under 16 years of organic inputs (i.e., NRS, NGN, NDP, and NTM) or untreated soil (C) were determined on sieved (<1 mm) air-dried soil by Walkley–Black dichromate digestion, and micro-Kjeldahl methods, respectively. Three random soil samples from each of three replicate plots of each treatment were collected at 0–15 cm depth using an auger with 2.5 cm diameter and bulked. The field-moist soils were air dried, sieved (<2 mm) with removal of visible organic debris and maintained at room temperature for 7 days until the incubation experiment was started.

Incubation experiments

Two incubation experiments were simultaneously performed. Experiment I was conducted to study the abundance and structure of fungal community and enzyme activities of soils as affected by long-term (16 years) yearly application of organic inputs contrasting in biochemical quality, i.e., N-soils (NRS, NGN, NDP, and NTM) and untreated C-soil (objective 1). Experiment II was conducted to study the response of enzyme activities to freshly added biochemically contrasting organic inputs (objective 2). For experiment II, locally available organic inputs were used. Rice straw (RS) and groundnut stover (GN) were collected from cultivated fields, while tree litter used were dipterocarp leaves (DP) as well as tamarind leaves and petiole (TM) from field trial adjacent forests. Organic materials were air-dried. Rice straw and groundnut stover were cut into pieces of 1 cm in length, while dipterocarp leaves were cut into a square shape of 1 × 1 cm. Tamarind was applied as whole leaves plus cut petioles (1 to 3 cm). The incubation experiments were set up in randomized complete block designs with three replicates for each treatment.

For both incubation experiments, six hundred grams (dry weight) of C-soil and each N-soil were put in 1-l jars (approx. 11 cm diameter and 13 cm height). The thickness of the soil in the jar was 4 cm. For experiment I, five treatments as indicated above were used while experiment

Table 1 Biochemical properties (quality) of organic inputs

Residue	Cellulose	N	Polyphenols [PP]	Lignin [L]	L/N ratio	PP/N ratio	(L+PP)/N ratio	C/N ratio
	$g\ kg^{-1}$							
Rice straw (RS)	507	4.7	6.5	28.7	6.1	1.4	7.5	78.4
Groundnut (GN)	178	22.8	12.9	67.6	2.96	0.6	3.5	17.1
Dipterocarp (DP)	306	5.7	64.9	175.5	30.8	11.4	42.2	79.5
Tamarind (TM)	143	13.6	31.5	87.7	6.4	2.3	8.8	31.5

Original data taken from Puttaso et al. (2011a)

Table 2 Selected properties of topsoil (0–15 cm) at initial stage and after 16 years of organic inputs of the field experiment

Parameters	Treatment						SED	Significance[c]
	Year 1[a]	Year 16[b]						
	C	C	NRS	NGN	NDP	NTM		
pH (H_20) (1:2.5)	5.50	5.08 d	5.90 c	6.05 b	5.90 c	6.52 a	0.046	***
Exchangeable Ca ($cmol_c\ kg^{-1}$)	–	0.14 c	0.34 bc	0.56 b	0.42 b	0.97 a	0.103	***
Total C ($g\ kg^{-1}$)	2.1	1.74 d	2.43 c	3.50 b	3.42 b	3.94 a	0.089	***
Total N ($g\ kg^{-1}$)	0.2	0.06 d	0.12 c	0.25 a	0.11 c	0.21 b	0.005	***
Soil C/N ratio	10.5	29.0 a	20.3 b	14.0 c	31.1 a	18.8 b	1.858	***

SED standard error of the difference

[a] Original data taken from Vityakon et al. (2000)

[b] Means in the same row followed by the same letter are not significantly different at $P \leq 0.05$ (LSD)

[c] LSD test at significance levels: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

II, the treatments were C-soil either treated with each organic input (i.e., C+RS, C+GN, C+DP, and C+TM) or left untreated (C). Applied organic input was thoroughly mixed with soils at the rate equivalent to 10 Mg ha^{-1} based on a soil depth of 0–15 cm (e.g., in the range of 2.42–2.67 g kg^{-1} soil dry weight [DW]). For each jar, initial soil water adjustment with distilled water to 60 % water holding capacity (WHC) and additional time to reach equilibrium took 3 h. The amount of water added to the soils on the percentage soil dry weight basis was for C (8.16 %), NRS (8.26 %), NGN (8.20 %), NDP (7.91 %), and NTM (8.32 %). During the incubation, jars were slightly opened to allow air exchange, kept at 25 °C and watered with distilled water as necessary to keep constant soil moisture. For experiment I, soil samples from treatments C, NRS, NGN, NDP, and NTM were obtained 3 h after incubation started which was later referred to as day 0. For experiment II, soil samples of C, C+RS, C+GN, C+DP, and C+TM treatments, were taken 3 h (to allow soil moisture adjustment) and 56 days (to allow for stable microbial activities) after incubation. Soil samples were frozen at −20 °C until further analyses.

Fungal community structure analysis

For studying the fungal community structure using molecular techniques, a portion of the frozen soil from incubation experiment I (i.e., C, NRS, NGN, NDP, and NTM) was freeze-dried and kept under dry conditions. Total soil genomic DNA was extracted using the Fast DNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) following the manufacturer's instructions with slight modifications. Briefly, 0.3 g freeze-dried soil was bead-beated for 30 s with a beating power of 5.5 using a FastPrep®-24 Instrument (MP Biomedicals). The washing step, which was repeated three times, included addition of one ml 5.5 M guanidine thiocyanate (Sigma-Aldrich, Munich, Germany) to the binding matrix suspension followed by centrifugation at 14,000×*g* for 5 s to remove humic substances from extracts. Extracted DNA was quantified photometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, DE, USA).

Quantification of 18S rRNA gene copy numbers in soils was performed using oligonucleotides FF390 (5′-CGA TAACGAACGAGACCT-3′) and FR1 (5′-AICCATT CAATCGGTAITCATTCA-3′) (Vanio and Hantula 2000). Each reaction (20 μl) contained 2.5 ng DNA template, 10 μl of SYBR® green (Applied Biosystems, Foster City, CA, USA), 0.2 μl T4 gene 32 protein (500 ng μl^{-1}, MP Biomedicals), and 0.2 μM of each oligonucleotide. A cloned amplicon was used as standard in 10-fold serial dilutions of known DNA concentration. PCR runs were performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Cycling started with initial denaturation at 95 °C for 10 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and polymerization at 70 °C for 1 min. Each DNA sample was processed in triplicate reactions, while standards were run in duplicates. Melting curve analysis of amplicons was conducted to confirm that fluorescence signals originated from specific amplicons and not from oligonucleotide dimers or other artifacts. A reaction efficiency of 98.6 % was achieved. Quantification of gene copies was calculated by comparing the values of threshold cycles (C_t) to the values of the crossing points of the linear regression line of the standard curve using StepOne™ software version 2.2 (Applied Biosystems).

The fungal community structure was studied by terminal restriction fragment length polymorphism (T-RFLP) analysis and generation of gene libraries using the same oligonucleotide set as was applied for quantitative PCR. For T-RFLP analysis, the 18S rRNA gene was amplified in 25-μl reactions containing 5 ng DNA template, 1× PCR buffer, 2 U Taq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 0.2 mM of each deoxynucleoside triphosphate, 0.2 μM of each oligonucleotide, and 1.0 mM $MgCl_2$. The reverse

oligonucleotide FR1 was labeled with the fluorescent dye FAM-6. PCRs were started with initial denaturation at 95 °C for 1 min, followed by 30 cycles consisting of a denaturation at 95 °C for 30 s, an annealing step at 52 °C for 45 s, and elongation at 72 °C for 2 min. The reactions were completed with a final elongation step at 72 °C for 10 min. Amplicons were purified using the illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Munich, Germany) following the manufacturer's instructions. For digestion, 200 ng of amplicons were incubated with 5 U *Msp*I restriction endonuclease (Promega GmbH, Mannheim, Germany) at 37 °C for 4 h followed by 65 °C for 20 min enzyme inactivation. *Msp*I-digested products were desalted with Sephadex™G-50 (GE Healthcare) and amended with 7.75 μl Hi-Di formamide (Applied Biosystems) and 0.25 μl internal size standard GeneScan™-500 ROX™ (Applied Biosystems). Mixtures were denaturated at 95 °C for 2 min, followed by immediate chilling on ice. T-RFLP profiles were recorded on an ABI Genetic Analyzer 3130 (Applied Biosystems). Peak Scanner software (version 1.0, Applied Biosystems) was used to compare relative lengths of terminal-restriction fragments (T-RFs) with the internal size standard and to compile electropherograms into numeric data sets, in which T-RF length and height >50 fluorescence units were used for statistical profile comparison. T-RFLP profiles used for statistical analyses were normalized according to Dunbar et al. (2000).

For phylogenetic assignment of T-RFs in T-RFLP profiles obtained, purified amplicons of the five assayed treatments were ligated into the Strata-Clone PCR cloning vector pSC-A (Stratagene, La Jolla, CA, USA), and ligation products were transformed with StrataClone SoloPack competent cells (Stratagene). Of each treatment, 40 positive colonies were randomly selected, M13-PCR performed, and partially sequenced with reverse oligonucleotide M13 (LGC Genomics GmbH, Berlin, Germany). Sequence information of approximately 350 bp per clone was subjected to GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) under the National Center for Biotechnology Information (NCBI). Obtained sequence information was deposited in Genbank under accession numbers JX268040 to JX268239.

Enzyme activity analysis

Samples from all treatments in both incubation experiments were used for enzyme activity analysis. Invertase (EC 3.2.1.26) was measured as described by Schinner and von Mersi (1990, cited in Alef and Nannipieri 1995) using 50 mM sucrose as substrate with 2 M acetate buffer (pH 5.5). Activity of invertase was expressed as mg glucose equivalent (GE) g^{-1} soil DW 3 h^{-1}. B-glucosidase (EC 3.2.1.21) activity was measured as described by Alef and Nannipieri (1995) using 25 mM *p*-nitrophenyl-β-D-glucopyranoside as substrate with modified universal buffer (MUB), pH6.0. Activity of B-glucosidase was expressed as μg *p*-nitrophenol released g^{-1} soil DW h^{-1}. Phenoloxidase (EC 1.10.3.1, 2) and peroxidase (EC 1.11.1.7) activities were measured as described by Hendel et al. (2005) using 5 mM L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate with 50 mM acetate buffer. For peroxidase activity, 0.3 % (v/v) hydrogen peroxide (H_2O_2) was used as electron acceptor. Activity of phenoloxidase was calculated using 1.66 μmol as the extinction coefficient for L-DOPA. Similar to phenoloxidase activity, activity of peroxidase was calculated as the difference in activity between samples treated with and without H_2O_2. Activities of phenoloxidase and peroxidase were expressed as μmol 2,3-dihydroindole-2-carboxylate (dicq) g^{-1} soil DW h^{-1}.

Statistical analysis

One factor analysis of variance (ANOVA) along with least significant differences (LSD) were used to analyze the main treatment effects on assayed soil microbial parameters ($P<0.05$) (SPSS for Windows, version 17.0; SPSS Inc., Chicago, IL, USA.). These statistical analyses were performed on soil chemical characteristics and 18S rRNA gene-based fungal abundance (quantitative PCR) and normalized T-RFLP fingerprints in incubation experiment I as well as for enzyme activities in both incubation experiments I and II. T-RFLP data sets were further analyzed on the basis of Bray–Curtis similarity coefficients (Legendre and Legendre 1998). Therefore, a similarity matrix was generated for all possible pairs of samples of each target group (i.e., C, NRS, NGN, NDP, and NTM). This similarity matrix was then used for analysis of similarity (ANOSIM) statistics (Clarke and Green 1988) to test if fungal communities were altered by long-term addition of biochemically contrasting organic inputs. ANOSIM generates a test statistics, *R*. The magnitude of *R* indicates the degree of separation between two microbial communities, with a score of 1 indicating complete separation and 0 indicating no separation. Treatment separation was further visualized by non-metric multidimensional scaling (nMDS). nMDS calculates a Kruskal's stress value indicating the fitness of similarity ranking, which should be less than 0.2 to warrant a justified treatment separation (Clark and Warwick 2001). Calculation of similarity coefficients, ANOSIM and nMDS were carried out using Primer for Windows version 6 (Primer-E Ltd., Plymouth, UK).

Results

Soil microbial community structure

Long-term addition of tamarind inputs produced the highest content of total C in the NTM soil followed by groundnut

inputs, whereas groundnut showed highest total N in the NGN treatment ($P<0.05$) (Table 2). NTM treatment had significantly highest pH (6.52, $P<0.05$) followed by NGN treatment (6.05, $P<0.05$) which was significantly higher than NRS, NDP and C treatments. The largest gene copy number was found in the NTM treatment (1.84E+09 copies g^{-1} soil DW, $P<0.05$) followed by the NGN treatment (4.91E+08 copies g^{-1} soil DW). Gene copies in the NRS and NDP were significantly smaller than that in NTM, but not in other input treatments or the control (C) (Fig. 1).

Data obtained from *Msp*I digestion-based T-RFLP analysis of fungal 18S rRNA genes in N- and C-soils was first studied by analysis of variance which revealed 11 T-RFs (i.e., 53, 86, 151, 152, 161, 210, 290, 305, 307, 308, and 310 bp) with significant ($P<0.05$) response to biochemically contrasting organic inputs as reflected in T-RF height differentiations (Table 3). ANOSIM determined distinct community differences between C- and N-soils. The largest community difference as compared to the C-soil was found in NDP ($R=0.926$) and NTM ($R=0.815$) treatments. Treatments NRS and NGN took intermediate positions showing R values of 0.407 and 0.444, respectively, in comparison to the unamended C-soil. These clear long-term effects of organic inputs on the soil fungal community structure were confirmed by nMDS showing clear separations between treatments with a substantiating stress value of 0.08 (Fig. 2).

A total of 200 (40 per each of the five analyzed treatments: C, NRS, NGN, NDP, and NTM) partial 18S rRNA gene sequences was subjected to BLAST analysis in the NCBI database (Tables 3 and 4). In all five gene libraries, the majority of sequences were affiliated with the phylum Ascomycota which was predominantly represented by sequences of *Myceliopthora thermophila* (all treatments (T-RF 151/152 bp), *Aspergillus versicolor* (NDP, and NTM [T-RF 151/152 bp]), *Fusarium oxysporum* (NTM [T-RF 151/152 bp]), *Anguillospora longissima* (NDP [T-RF 161 bp]), *Cladosporium bruhnei* (C [T-RF 290 bp]), and *Aspergillus fumigatus* (NGN, and NDP [T-RF 305–310 bp]). In the NTM treatment, several sequences were, in contrast to the others, assigned as *Cryptococcus podzolicus* (Basidiomycota; T-RF 305–310 bp). Several sequences were affiliated with protozoa with large proportions in treatment NGN such as the predominantly found *Proleptomonas faecicola* (Rhizaria; T-RF 151–152 bp).

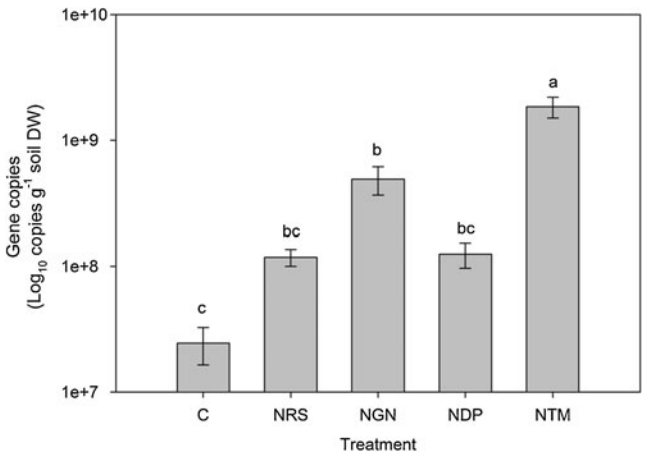

Fig. 1 Quantification of 18S rRNA genes in native soils treated with different organic inputs (rice straw [*NRS*], groundnut stover [*NGN*], as well as leaf litter from dipterocarp [*NDP*], and tamarind [*NTM*]) and control soil without any input (*C*) for 16 years. Presented data (18S rRNA gene copies per gram dry soil) are average values calculated from three individual soil samples per treatment. Different letters indicate significant differences at $P<0.05$. Bars represent standard deviations of three measurements

Enzyme activity analysis

Activities of invertase, B-glucosidase, phenoloxidase, and peroxidase showed different long-term responses to organic inputs (Fig. 3) (Experiment I). Invertase activity was generally increased in N-soils as compared to C-soil, but a significant difference was only found between untreated C-soil (0.25 mg GE g^{-1} soil DW 3 h^{-1}), NDP (0.51 mg GE g^{-1} soil DW 3 h^{-1}), and NTM (0.57 mg GE g^{-1} soil DW 3 h^{-1}) ($P<0.05$). B-glucosidase activity was only significantly higher in NGN (58.78 μg *p*-nitrophenol g^{-1} soil DW h^{-1}) and NTM (59.42 μg *p*-nitrophenol g^{-1} soil DW h^{-1}) soils as compared to the control (33.42 μg *p*-nitrophenol g^{-1} soil DW h^{-1}) ($P<0.05$). NDP was the only treatment under which phenoloxidase activity was significantly enhanced over the control (0.08 dicq g^{-1} soil DW h^{-1}) ($P<0.05$). NRS showed significantly higher peroxidase activity as compared to control (0.5 dicq g^{-1} soil DW h^{-1}) and NTM (0.42 dicq g^{-1} soil DW h^{-1}) ($P<0.05$). In addition, NRS as well as control treatments showed significantly ($P<0.05$) higher specific peroxidase activity (activity expressed per unit of total C in soil) (0.29 μmol dicq mg^{-1} C h^{-1}) than the other residue treatments (0.11–0.16 μmol dicq mg^{-1} C h^{-1}).

Upon addition of fresh organic inputs to the control soil (Experiment II), invertase activities were significantly higher at day 0 than day 56 only under C+RS (0.40 versus 0.14 mg GE g^{-1} soil DW 3 h^{-1}), C+GN (0.51 versus 0.29 mg GE g^{-1} soil DW 3 h^{-1}), and control (0.25 versus 0.09 mg GE g^{-1} soil DW 3 h^{-1}) ($P<0.05$) (Fig. 4). Similar response patterns to the input treatments were shown for both sampling times at day 0 and 56. The highest enzyme activity was found in C+GN (0.51 mg GE g^{-1} soil DW 3 h^{-1}) followed by C+RS (0.40 mg GE g^{-1} soil DW 3 h^{-1}). No significant difference was found between C, C+DP and C+TM ($P>0.05$). B-glucosidase activity showed for all treatments no significant difference between the two sampling dates ($P>0.05$) (Fig. 4). At day 0, no significant differences were found among treatments. On the other

Table 3 Terminal restriction fragments (T-RFs) generated from *Msp*I digestion which were significantly affected by 16 years of continuous application of biochemically contrasting organic inputs as determined by analysis of variance

Actual T-RF size (bp)	Theoretical T-RF size (bp)[a]	Significance level[b]	Affiliation of predominant (n>5) fungal and protozoan clones to individual T-RFs[c]				
			C	NRS	NGN	NDP	NTM
53	–	*					
86	–	*					
151/152	156/157	***/**	*Myceliopthora thermophila*	*Myceliopthora thermophila*	*Myceliopthora thermophila; Proleptomonas faecicola*	*Myceliopthora thermophila; Aspergillus versicolor*	*Myceliopthora thermophila; Aspergillus versicolor; Fusarium oxysporum*
161	165	*				*Anguillospora longissima*	
210	–	***					
290	294–298	**	*Cladosporium bruhnei*				
305/307/308/310	312/315	*/***			*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Cryptococcus podzolicus*

[a] Theoretical T-RF sizes were only counted if generated clones could be clearly assigned to a respective T-RF size

[b] ANOVA T-RF height vs. organic input treatments. LSD test at significance levels: *P<0.05, **P<0.01, ***P<0.001

[c] Genbank accession numbers of listed fungal and protozoan species are given in Table 3

hand, at day 56, significantly higher B-glucosidase activities were found in C+RS (47.39 μg *p*-nitrophenol g^{-1}soil DW h^{-1}) and C+GN (48.35 μg *p*-nitrophenol g^{-1} soil DW h^{-1}) than the control (33.42 μg *p*-nitrophenol g^{-1} soil DW h^{-1}). For phenoloxidase activity, no significant difference was detected for all treatments at day 0. At day 56, significantly highest phenoloxidase activity was measured in C+DP (0.67 dicq g^{-1} soil DW h^{-1}), while those of C+GN (0.43 dicq g^{-1} soil DW h^{-1}) and C+TM (0.35 dicq g^{-1} soil DW h^{-1}) were higher against the control (0.13 dicq g^{-1} soil DW h^{-1}) (P<0.05). For C+GN, C+DP, and C+TM, phenoloxidase activity was highly promoted at day 56 as compared to day 0 (P<0.05). Peroxidase activity, at both sampling dates, was not significantly affected by treatments (P>0.05) with the exception of at day 0 under C+DP which was significantly lower than C+GN and C+TM treatments. In all treatments, with the exception of C+DP, activities of peroxidase were lower in day 56 than day 0.

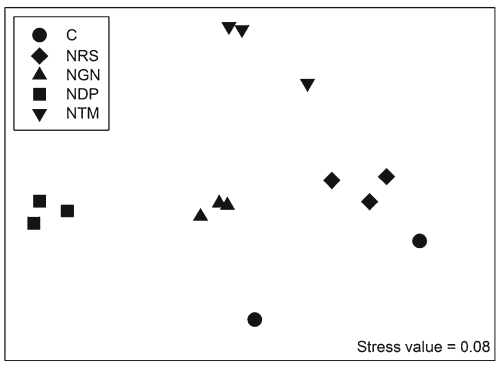

Fig. 2 Bray–Curtis similarity-based non-metric multidimensional scaling plot of normalized T-RFLP data obtained from *Msp*I-digested 18S rRNA gene amplicons visualizing the distinct differences of fungal communities in soils treated with biochemically contrasting organic inputs for 16 years. Treatments are: control soil without any input (*C*), rice straw (*NRS*), groundnut stover (*NGN*), as well as leaf litter from dipterocarp (*NDP*), and tamarind (*NTM*)

Discussion

The biochemical quality of organic inputs has the potential to regulate fungal community structures and their catabolic capabilities in the long term. In this regard, we could show that recalcitrant, thus complex organic compounds including lignin and polyphenols, and more labile substances (e.g., cellulose) and N, were major determinants of fungal abundance and community composition which, in turn, contributed to soil microbial functioning as a whole including bacteria and protozoa.

It was particularly evident that the long-term incorporation of dipterocarp litter caused a distinct difference in the fungal community composition as compared to the untreated control soil, but on the other hand, abundance of fungi based on quantified 18S rRNA gene copies was not different from other treatments with the exception of the tamarind treatment. We explained this response of 16 years dipterocarp litter addition by its particularly high contents of lignin and polyphenols which seemed to restrict the fungal communities to a narrow species spectrum specialized in the decomposition of these recalcitrant compounds (Mutabaruka et al. 2007). In contrast, the determined community differences suggested that these recalcitrant compounds of dipterocarp may have promoted specifically phenoloxidase expressing fungal decomposers.

Table 4 Phylogenetic assignment of gene libraries of amplified 18S rRNA genes of control soil without any organic input (C) and N-soils treated with rice straw (NRS), groundnut stover (NGN), as well as leaf litter from dipterocarp (NDP), and tamarind (NTM) for 16 years (approximately 350-bp sequence information per clone)

Closest NCBI match (accession number)/% homology	Corresponding clone	Clone number per treatment of C- and N-soils				
		C	NRS	NGN	NDP	NTM
Ascomycota						
Anguillospora longissima (AY204599)/100	CloNRS04; CloNDP01/04/05/07/22/26/29/41		1		8	
Aspergillus fumigatus (HQ871893)/99–100	CloNGN04/07/12/13/26–28/36/37			12	12	
	CloNGN43–45; CloNDP02/03/08/09/13/16/24; CloNDP25/34/37/42/51					
Aspergillus oryzae (JF265070)/99	CloC14	1				
Aspergillus versicolor (GU227343)/99–100	CloNDP17/19/21/23/27/28/32/35/36				10	5
	CloNDP47; CloNTM42/43/44/45/52					
Bahusakala longispora (GQ280423)/95–99	CloNGN23; CloTM27/29			1		2
Cladophialophora sp.CBS 985.96 (AJ232953)/100	CloNTM02/21/26					3
Cladosporium bruhnei (JN397376)/100	CloC03/05–08/12/52/57	8				
Cochliobolus cynodontis (JN941646)/99)	CloNRS15; CloNGN03/40; CloNDP43/48		1	2	2	
Cochliobolus kusanoi (JN941641)/99	CloNGN24			1		
Corynespora cassiicola (GN296145)/99	CloNDP50				1	
Didymocrea sadasivanii (DQ384066)/98	CloNRS13		1			
Fusarium oxysporum (JF807401)/99–100	CloNTM07/38/46–48/55					6
Myceliopthora thermophila (CP003008)/99–100	CloC09/17–20/22/24/25/28/29/32/34	23	29	10	4	5
	CloC35/38–41/43/48/51/53/56/58					
	CloNRS06/10/11/14/17/20–23					
	CloNRS25–27/29–32/34–45/47; CloNGN09/15/16/19/25/34/38/39/46CloNGN49; CloNDP 11/14/20/31; CloNTM22/25/30/32/51					
Neurospora crassa (FJ610444)/99–100	CloNRS05/24; CloNGN32		2	1		
Ochroconis humicula (AB600877)/100	CloNRS08		1			
Ophiocordyceps prolifica (JN941708)/99	CloC01	1				
Phaeodothis winteri (DQ678021)/100)	CloC21	1				
Phoma macrostoma (AB454217)/100	CloC46	1				
Phoma sp. MA 4794 (AJ972796)/99	CloNDP12/46				2	
Simplicillium lanosoniveum (HQ232185)/100	CloC47	1				
Basidiomycota						
Pholiota multicingulata (HQ832430)/97–99	CloC31/54	2				
Cryptococcus podzolicus (AB032645)/98–99	CloNGN11; CloNTM09/12/15/17/19			1		13
	CloNTM20/31/40/41/49/50/53/54					
Zygomycota						
Rhizopus oryzae (JN003654)/100	CloNDP45				1	
Blastocladiomycota						
Catenomyces sp. JEL 342 (AY635830)/99	CloNGN35			1		
Alveolata (protozoa)						
Uncultured *Eimeriidae* clone AMB_18s_667 (EF023344)/96	CloC37	1				
Uncult. *Eimeriidae* clone ELV_18s_1051 (EF024395)/99	CloNTM03–06/33/34					6
Rhizaria (protozoa)						
Proleptomonas faecicola (GQ377682)/92	CloC27; CloNGN01/02/05/06/10/14	1		11		
	CloNGN20/22/29/30/31					
Uncult. cercozoan clone Sey 012 (AY605185)/93	CloNRS03		1			
Glissomonad sp. Panama 51 (EU709219)/98	CloNRS19		1			
Spongomonas minima (AF411280)/90	CloNRS16		1			
Unclassified organisms						
Uncult. Clone Nabaos_u_73 (DQ865555)/99	CloNRS28/33		2			

NCBI National Center for Biotechnology Information

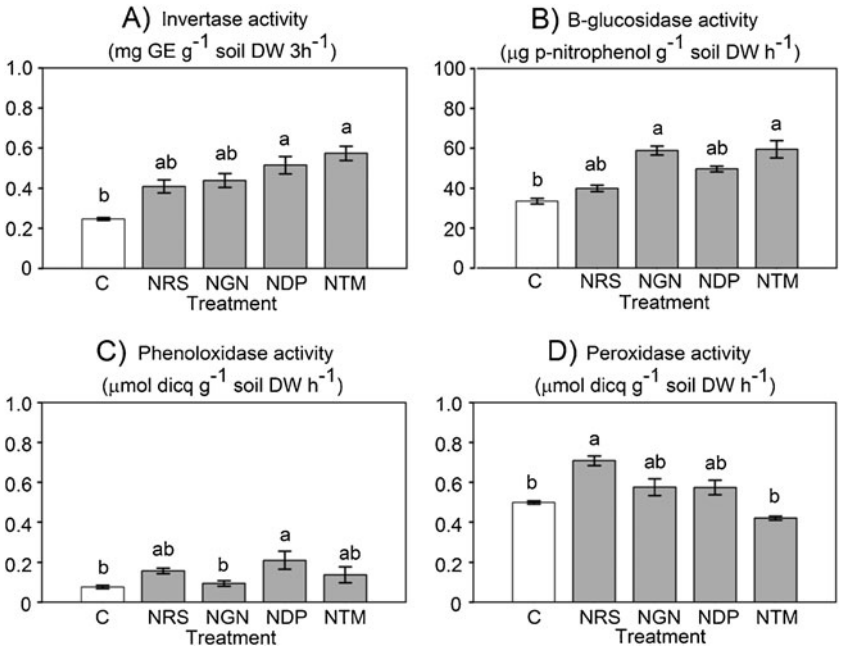

Fig. 3 Invertase (**a**), B-glucosidase (**b**), phenoloxidase (**c**), and peroxidase (**d**) activities in incubated soil samples after 16 years of residue addition. Treatments are: control soil without any input (*C*), rice straw (*NRS*), groundnut stover (*NGN*), as well as leaf litter from dipterocarp (*NDP*), and tamarind (*NTM*) at day 0 incubation. Presented data are average values calculated from three analytical replicates per treatment. Different letters indicate significant differences at $P<0.05$. Bars represent standard error of means of three measurements

Expression of phenoloxidase was particularly responded to the polyphenol-rich compounds treated soil from the long-term dipterocarp treatment. Additionally, interaction between contents of N and polyphenols of organic inputs seemed to regulate the expression of phenoloxidase. This was seen in the lower polyphenol/N ratio than dipterocarp of tamarind>rice straw>groundnut stover. This assumption was substantiated by the increase of phenoloxidase activity in the long-term under 16 yearly applications of dipterocarp. In addition, the generated 18S rRNA gene libraries have shown that the dominant *Anguillospora longissima* which may have been, among others, responsible for the distinct increase of phenoloxidase activity as previously shown by Abdullah and Taj-Aldeen (1989).

A similar difference of the fungal community composition between NTM ($R=0.815$) and NDP ($R=0.926$) relative to the control were induced by long-term addition of tamarind inputs. The significant increase of 18S rRNA gene abundance in the NTM treatment was induced by the highest C accumulation associated with small macroaggregates formation (Samahadthai et al. 2010). Another potential explanation for this was the high N content of tamarind inputs applied annually promoting the protection and accumulation of N through their chemical stabilization (polyphenol–

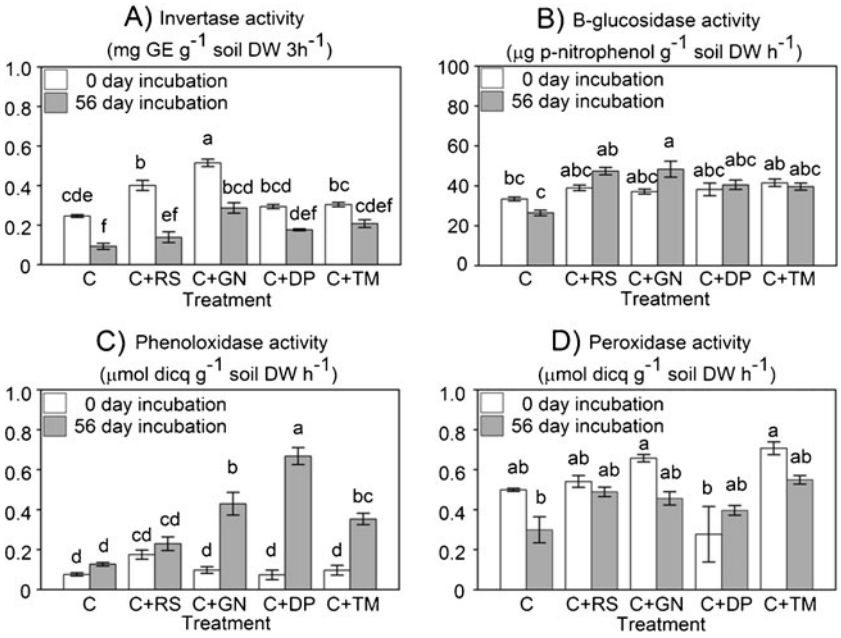

Fig. 4 Invertase (**a**), B-glucosidase (**b**), phenoloxidase (**c**), and peroxidise (**d**) activities in incubated control soil samples (*C*) with or without fresh input addition. Treatments are: control soil without any input (*C*), rice straw (*C+RS*), groundnut stover (*C+GN*), as well as leaf litter from dipterocarp (*C+DP*), and tamarind (*C+TM*) at day 0 and 56 incubation. Presented data are average values calculated from three analytical replicates per treatment. Different letters indicate significant differences at $P<0.05$. Bars represent standard error of means of three measurements

protein complexation) (Mutabaruka et al. 2007) within aggregates (Liao et al. 2006).

The resulting high fungal abundance and organic matter may have contributed to the enhancement of the potential activities of invertase and B-glucosidase as consequence of higher N supply (Puttaso et al. 2011a). This was particularly reported for NTM and NGN treatments as was supported by Sjöberg et al. (2004). These authors found enhanced cellulose decomposition in N-rich litter. Although it could not be ruled out that bacteria have contributed to cellulose decomposition in the studied soil systems, we found further evidence for the high potential activities of these two enzymes through the presence of *Fusarium oxysporum* (Sampredo et al. 2007). This species is known as a dominant member of the fungal community with reported abilities to produce B-glucosidase to decompose labile cellulose in the NTM soil. Additionally, *Cryptococcus podzolicus*, another dominant species found in this treatment has also been reported to utilize labile sugars (i.e., glucose, and sucrose) and short-chain cellulose (Fonseca et al. 2011).

We hypothesize that this decomposition stimulation was also the consequence of aggregate-associated organic compounds, such as residual sugars, particularly produced by soil fungi. This hypothesis was supported by the findings of increased aggregate formation in a coarse-textured (72 % sand) soil mediated by mucilage (saccharides)-producing fungi (Caesar-TonThat 2002). As support for this, long-term application of tamarind was shown to promote the formation of microaggregates with subsequent accumulation of organic C and associated protection of labile C compounds (Bastida et al. 2012; Lagomarsino et al. 2012; Verchot et al. 2011). This accumulation of organic C might have therefore explained the significantly lower peroxidase activity in the NTM than in the NRS soil as a consequence of the higher remaining availability of easily decomposable C substrates associated with aggregates in the NTM than the NRS soils. Under these circumstances, certain fungi may not be forced to switch their metabolism from low energy demanding processes such as B-glucosidase synthesis to that of energetically costly peroxidase synthesis (Qin et al. 2010). In contrast to NTM, there was in the NRS treatment a trend of lower invertase and B-glucosidase activities which indicated that the soil fungi may have been deprived of easily available C resources. Additionally, the significantly higher specific peroxidase activity (enzyme activity per unit of C inputs) in the NRS relative to the other organic input soils indicated further that the quality of soil organic C retained under the NRS treatment was relatively more recalcitrant in nature than the NTM which induced the synthesis of peroxidase. This is further substantiated by the results of the lowest soil organic C accumulation under long-term rice straw treatment in the field experiment indicating a potentially complete decomposition of labile C substrates at earlier stages after rice straw addition as well as a priming of native organic matter due to limited N supply (Puttaso et al. 2011a).

A major proportion of the microbial (fungal) community is functionally redundant (Nannipieri et al. 2003; Rasche and Cadisch 2013). On the other hand, environmental stress such as soil pH and deprivation of organic matter input may regulate the diversity and functional potential of soil microbial communities (Chaparro et al. 2012; Nannipieri et al. 2012). Rousk et al. (2010) have reported that organic residue derived alteration in soil pH has profound short-term effects on composition of soil microbial community, i.e., bacteria versus fungi. They observed that bacterial growth was positively related to an increase in soil pH, while the opposite was true for fungal growth. Long-term yearly application of contrasting quality organic inputs in this study have created specific environment as shown by differing soil pH under these organic input treatments which was to have significant impact on microbial diversity and functioning. In this sense, the biochemical quality of organic input may promote functionally specialized phyla or even species not only a soil fungal community but also bacteria and other microorganisms. Contrastingly, the communities that remained dormant, with potentially redundant functional capabilities (e.g., B-glucosidase) may be regenerated, while others that were active may switch their metabolism to capitalize on the change in substrate availability and quality. The latter became particularly evident for studied fungi in the control soils which have been treated with the four biochemically contrasting organic inputs resulting in increases in enzyme activities (e.g., increased phenoloxidase activity in unamended control soil with freshly added dipterocarp). In Experiment I, the degree of functional redundancy became particularly visible for invertase and B-glucosidase activities, which were induced (e.g., by a potential switch of metabolism or re-activation of soil bacteria) after the addition of easily decomposable rice straw and groundnut stover. This supported the findings by Chigineva et al. (2009), Zhao et al. (2005), and Schutter and Dick (2001) on the dominant fungal community being reactivated by labile C compounds. In our case, *M. thermophila* was the dominant community member with reported metabolic capabilities to decompose cellulose (Roy et al. 1989) and also phenolic compounds (Babot et al. 2011). In addition, the potentially functional specialization (i.e., phenoloxidase synthesis) of certain members of the soil fungal community was obvious in the control soils treated with dipterocarp litter. This interesting result was supported by the acknowledged capabilities of *M. thermophila* to produce laccase (Babot et al. 2011). In addition, the dominance of *Cladosporium bruhnei* in the control soil indicated that members of the genus *Cladosporium* sp. have obvious capabilities in decomposing cellulolytic compounds under oligotrophic conditions (Ghahfarokhi et al. 2004). However, it should be noted that

the chosen procedure of using air-dried soils for the incubation experiments could be a factor affecting the assessment and interpretation of microbial community dynamics and soil enzyme activities as affected by substrate quality of organic inputs in the present study, in which only potential and not actual activities were measured (Nannipieri et al. 2012).

Surprisingly, except for the NDP treatment, libraries of amplified 18S rRNA genes using the oligonucleotide set FR1::FF390 revealed several clones affiliated to non-fungal eukaryotic organisms. In particular, the presence of protozoa was noted. The occurrence of protists during decomposition of organic inputs was recently recognized by Murase et al. (2012). In this regard, the occurrence of Rhizaria (e.g., *Proleptomonas faecicola*) was most pronounced in the NGN treatment, while sequences related to the Alveolata phylum were found in the NTM treatment. This interesting result showed the obvious dependence of protists on N availability resulting from N mineralization as both N input types increased the N level in the respective soil systems (Puttaso et al. 2011a). It was earlier confirmed by Kuikman et al. (1990, 1991) that there were close interactions between protozoa and bacteria as both are involved in N mineralization and SOM turnover. Similar interactions with fungi in the studied soil systems, however, are subject to prospective research. Although the used oligonucleotide set was designed for a broad range of fungal 18S rDNA targets and proved to show high accuracy for abundance and phylogenetic studies of fungal communities (Vanio and Hantula 2000) over other oligonucleotide sets with comparable drawbacks which were recently evaluated by Anderson et al. (2003) and Chemidlin Prévost-Bouré et al. (2011). It was also shown by Hoshino and Matsumoto (2007) and Hagn et al. (2003) that the sequence analysis of amplified 18S rDNA using the FF390::FR1 set contained sequences affiliated with Viridiplantae, Cercozoa, and Metazoa.

Conclusions

Our results pointed out that biochemical composition of organic inputs are key factors regulating the soil microbial, but particularly fungal community structure and their decomposition functions, both in the long and short term. In the short term, it was particularly evident by the studied enzyme activities that their increases occurred as reflected by functionally redundant and specialized reactions of the soil microbial community. The functional specialization was particularly obvious for the fungal community. Here, a stimulation of phenoloxidase activity was led back by recalcitrant, but low N containing organic inputs. In this sense, we could further show that substrate-dependent N availability either promoted or inhibited specific microbial (fungal) functions. This supports the ongoing controversial discussion on how N actually determines soil fungal communities and regulates their respective functions (e.g., Edwards et al. 2011; Keeler et al. 2009; Wu et al. 2011). This may also account for the presence of protozoa for which indications were found that these were regulated by the availability of N organic compounds in the studied soil systems. Prospective research should thus consider, apart from studying decomposing fungi, also protozoa and bacteria. This may include, apart from phylogenetic studies, those of functional genes (e.g., fungal genes encoding laccase or bacterial genes encoding proteolysis) to identify microbial (i.e., fungi, and bacteria) community members directly involved in the degradation of organic materials in soils as regulated by organic resource quality and environmental alterations such as changing SOM content and pH (Nannipieri et al. 2012; Rasche and Cadisch 2013; Rousk et al. 2010).

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References

Abdullah SK, Taj-Aldeen SJ (1989) Extracellular enzymatic activity of aquatic and aero-aquatic conidial fungi. Hydrobiologia 174:217–223

Abro S, Tian X, You D, Ba Y, Li M, Wu F (2011) Influence of microbial inoculants on soil response to properties with and without straw under different temperature regimes. Afr J Microbiol Res 4:3054–3061

Aciego Pietri JC, Brookes PC (2009) Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. Soil Biol Biochem 41:1396–1405

Alef K, Nannipieri P (1995) B-glucosidase activity. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, London, pp 350–352

Anderson IC, Campbell CD, Prosser JI (2003) Potential bias on fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environ Microbiol 5:36–47

Babot ED, Rico A, Rencoret J, Kalum L, Lund H, Romero J, del Río JC, Martínez AT, Gutiérrez A (2011) Towards industrially-feasible delignification and pitch removal by treating paper pulp with *Myceliopthora thermophila* laccase and a phenol mediator. Bioresour Technol 102:6717–6722

Bastida F, Jindo K, Moreno JL, Hernández T, Garcia C (2012) Effects of organic amendments on soil carbon fractions, enzyme activity and humus–enzyme complexes under semi-arid conditions. Eur J Soil Biol 53:94–102

Bierke A, Kaiser K, Guggenberger G (2008) Crop residue management effects on organic matter in paddy soils—the lignin component. Geoderma 146:48–57

Bissett A, Richardson AE, Baker G, Thrall PH (2011) Long-term land use effects on soil microbial community structure and function. Appl Soil Ecol 51:66–78

Caesar-TonThat TC (2002) Soil binding properties of mucilage produced by a basidiomycete fungus in a model system. Mycol Res 106:930–937

Caesar-TonThat TC, Cochran VL (2001) Role of saprophytic basidiomycete soil fungus in aggregate stability. In: Stott DE, Mohtar RH, Steinhardt GC (eds) Sustaining the global forum, 10th International Soil Conservation Organisation Symposium on Soil Erosion Research. Pardue University, West Lafayette, pp 575–579

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499

Chemidlin Prévost-Bouré N, Christen R, Dequiedt S, Mougel C, Lelièvre M, Jolivet C, Shahbazkia HR, Guillou L, Arrouays D, Ranjard L (2011) Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR. PLoS One 6:e24166. doi:10.1371/journal.pone.0024166

Chigineva NI, Aleksandrova AV, Tiunov AV (2009) The addition of labile carbon alters litter fungal communities and decreases litter decomposition rates. Appl Soil Ecol 42:264–270

Clark KR, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. Primer-E, Plymouth

Clarke KR, Green RH (1988) Statistical design and analysis of a biological effect study. Mar Ecol Prog Ser 46:213–226

Debosz K, Rasmussen PH, Pedersen AR (1999) Temporal variations in microbial biomass C and cellulolytic enzyme activity in arable soils: effects of organic matter input. Appl Soil Ecol 13:209–218

Dunbar J, Ticknor LO, Kuske CR (2000) Assessment of microbial diversity in four Southwestern United States soils by 16S rRNA gene terminal restriction fragment analysis. Appl Environ Microbiol 66:2943–2950

Edwards IP, Zak DR, Kellner H, Eisenlord SD, Pregitzer KS (2011) Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. PLoS One 6(6):e20421. doi:10.1371/journal.pone.0020421

España M, Rasche F, Kandeler E, Brune T, Rodriguez B, Bending GD, Cadisch G (2011) Assessing the effect of organic residue quality on active decomposing fungi in a tropical Vertisol using ^{15}N-DNA stable isotope probing. Fungal Ecol 4:115–119

Fonseca A, Boekhout T, Fell JW (2011) *Cryptococcus* Vuillemin (1901). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomy study, vol. 5. Elsevier, London, pp 1661–1737

Ghahfarokhi MS, Fazal A, Lotfi A, Abyaneh MR (2004) Cellobiose dehydrogenase production by the genus *Cladosporium*. Iran Biomed J 8:107–111

Hadas A, Kautsky L, Goek M, Kara EE (2004) Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol Biochem 36:255–266

Hagn A, Pritsch K, Ludwig W, Schloter M (2003) Theoretical and practical approaches to evaluate suitable primer sets for the analysis of soil fungal communities. Acta Biotechnol 23:373–381

Hendel B, Sinsabaugh RL, Marxsen J (2005) Lignin-degrading enzymes: phenoloxidase and peroxidase. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition: a practical guide. Springer, Dordrecht, pp 273–278

Hoshino YT, Matsumoto N (2007) Changes in fungal community structure in bulk soil and spinach rhizosphere soil after chemical fumigation as revealed by 18S rDNA PCR-DGGE. Soil Sci Plant Nutr 53:40–55

Keeler BL, Hobbie SE, Kellog LE (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

Kelly JJ, Bansal A, Winkelman J, Janus LR, Hell S, Wencel M, Belt P, Kuehn KA, Rier ST, Tuchman NC (2010) Alteration of microbial communities colonizing leaf litter in a temperate woodland stream by growth of trees under conditions of elevated atmospheric CO_2. Appl Environ Microbiol 76:4950–4959

Kuikman PJ, Jansen AG, Van Veen JA, Zehnder AJB (1990) Protozoan predation and the turnover of soil organic carbon and nitrogen in the presence of plants. Biol Fertil Soils 10:22–28

Kuikman PJ, Jansen AG, Van Veen JA (1991) ^{15}N-nitrogen mineralization from bacteria by protozoan grazing at different soil moisture regimes. Soil Biol Biochem 23:193–200

Kuzyakov Y, Bol R (2006) Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol Biochem 38:747–758

Lagomarsino A, Grego S, Kandeler E (2012) Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. Pedobiologia 55:101–110

Legendre P, Legendre L (1998) Numerical ecology, 2nd English edn. Elsevier, Amsterdam

Liao JD, Boutton TW, Jastrow JD (2006) Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biol Biochem 38:3184–3196

Lucas RW, Casper BB, Jackson JK, Balser TC (2007) Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands. Soil Biol Biochem 39:2508–2519

Luxhøi J, Magid J, Tscherko D, Kandaler E (2002) Dynamics of invertase, xylanase and coupled quality indices of decomposing green and brown plant residues. Soil Biol Biochem 34:501–508

Moscatelli MC, Lagomarsino A, Garzillo AMV, Pignataro A, Grego S (2012) β-glucosidase kinetic parameters as indicators of soil quality under conventional and organic cropping systems applying two analytical approaches. Ecol Indic 13:322–327

Murase J, Shibata M, Lee CG, Watanabe T, Asakawa S, Kimura M (2012) Incorporation of plant residue-derived carbon into the microeukaryotic community in a rice field soil revealed by DNA stable-isotope probing. FEMS Microbiol Ecol 79:371–379

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 Biol Biochem 39:1479–1492

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Fornasier F, Moscatelli MC, Marinari S (2012) Soil enzymology: classical and molecular approaches. Biol Fertil Soils 48:743–762

Nicolardot B, Bouziri L, Bastian F, Ranjard L (2007) A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities. Soil Biol Biochem 39:1631–1644

Palm CA, Gachengo CN, Delve RJ, Cadisch G, Giller KE (2001) Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource base. Agric Ecosyst Environ 83:27–42

Puttaso A, Vityakon P, Saenjan P, Trelo-ges V, Cadisch G (2011a) Relationship between residue quality, decomposition patterns, and

soil organic matter accumulation in a tropical sandy soil after 13 years. Nutr Cycl Agroecosyst 89:159–174

Puttaso A, Vityakon P, Trelo-ges V, Saenjan P, Cadisch G (2011b) Effect of long-term (13 years) application of different quality plant residues on soil organic carbon and soil properties of a sandy soil of Northeast Thailand. KKU Res J 16:359–370

Qin S, Hu C, He X, Dong W, Cui J, Wang Y (2010) Soil organic carbon, nutrients and relevant enzyme activities in particle-size fractions under conservational versus traditional agricultural management. Appl Soil Ecol 45:152–159

Rasche F, Cadisch G (2013) The molecular microbial perspective of organic matter turnover and nutrient cycling in tropical agroecosystems—what do we know? Biol Fertil Soils. doi:10.1007/s00374-013-0775-9

Roldán A, Salinas-García JR, Alguacil MM, Díaz E, Caravaca F (2005) Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions. Geoderma 129:178–185

Rousk J, Brookes PC, Bååth E (2010) Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biol Biochem 42:926–934

Roy SK, Raha SK, Dey SK, Chakrabarty SL (1989) Immobilization of β-glucosidase from *Myceliopthora thermophila* D-14. Enzym Microb Technol 11:431–435

Řzáčová V, Baldrian P, Hrselova H, Larsen J, Gryndler M (2007) Influence of mineral and organic fertilization on soil fungi, enzyme activities and humic substances in a long-term field experiment. Folia Microbiol 52:415–421

Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer Saccharum* forest soil. Soil Biol Biochem 34:1309–1315

Sajjad MH, Lodhi A, Azam F (2002) Changes in enzyme activity during the decomposition of plant residues in soil. Pak J Biol Sci 5:952–955

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 Degrad Dev 21:463–473

Sampredo I, D'Annibale A, Ocampo JA, Stazi SR, García-Romera I (2007) Solid-state cultures of *Fusarium oxysporum* transform aromatic components of olive-mill dry residue and reduce its phytotoxicity. Bioresour Technol 98:3547–3554

Schinner F, von Mersi W (1990) Xylanase-, CM-cellulase-, and invertase activity in soil: an improved method. Soil Biol Biochem 22:511–515

Schutter ME, Dick RP (2001) Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biol Biochem 33:1481–1491

Sinsabaugh RL, Gallo ME, Lauber C, Waldrop M, Zak DR (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 SM, de Moraes Sa JC, Albrecht A (2002) Soil organic matter, biota and aggregation in temperate and tropical soils-effects of no-tillage. Agronomie 22:755–775

Sjöberg G, Nilsson SI, Persson T, Karlsson P (2004) Degradation of hemicellulose, cellulose and lignin in decomposing spruce needle litter in relation to N. Soil Biol Biochem 36:1761–1768

Soil Survey Staff (2006) Key to soil taxonomy, 10th edn. USDA Natural Resources Conservation Service, Washington

Stemmer M, Gerzabek MH, Kandeler E (1999) Invertase and xylanase activity of bulk soil and particles-size fractions during maize straw decomposition. Soil Biol Biochem 31:9–18

Swift MJ, Heal OW, Anderson JM (1979) Influence of the physicochemical environment on decomposition process. In: Anderson DJ, Greig-Smith P, Pitelka FA (eds) Decomposition in terrestrial ecosystems. Studio in ecology, vol. 5. University of California Press, Berkeley, pp 220–266

Vanio EJ, Hantula J (2000) Direct analysis of wood-inhibiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. Mycol Res 104:927–936

Verchot LV, Dutaur L, Shepherd KD, Albrecht A (2011) Organic matter stabilization in soil aggregates: understanding the biogeochemical mechanisms that determine the fate of carbon inputs in soils. Geoderma 161:182–193

Vityakon P (2007) Degradation and restoration of sandy soils under different agricultural land uses in northeast Thailand: a review. Land Degrad Dev 18:567–577

Vityakon P, Dangthaisong N (2005) Environmental influences on nitrogen transformation of different quality tree litter under submerged and aerobic conditions. Agrofor Syst 63:225–236

Vityakon P, Meepech S, Cadisch G, Toomsan B (2000) Soil organic matter and nitrogen transformation mediated by plant residues of different qualities in sandy acid upland and paddy soils. Neth J Agric Sci 48:75–90

Wardle DA, Giller KE (1996) The quest for a contemporary ecological dimension to soil biology. Soil Biol Biochem 28:1549–1554

Wardle DA, Yeates GW, Nicholson KS, Bonner KI, Watson RN (1999) Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period. Soil Biol Biochem 31:1707–1720

Wu L, Feinstein LM, Valverde-Barrantes O, Kershner MW, Leff LG, Blackwood CB (2011) Placing the effects of leaf litter diversity on saprophytic microorganisms in the context of leaf type and habitat. Microb Ecol 61:399–409

Yuste JC, Penuelas J, Estiarte M, Garcia-Mas J, Mattana S, Ogaya R, Pujol M, Sardans J (2011) Drought-resistant fungi control soil organic matter decomposition and its response to temperature. Glob Chang Biol 17:1475–1486

Zhao Y, Li W, Zhou Z, Wang L, Pan Y, Zhao L (2005) Dynamics of microbial community structure and cellulolytic activity in agricultural soil amended with two biofertilizers. Eur J Soil Biol 41:21–29