Diversity of Fungi on Decomposing Leaf Litter in a Sugarcane Plantation and Their Response to Tillage Practice and Bagasse Mulching: Implications for Management Effects on Litter Decomposition

By Toshiko Miura; Ainin Niswati; I. G. Swibawa; Sri Haryani; Heru Gunito; Satoshi Shimano; Koichi Fujie; Nobuhiro Kaneko

WORD COUNT 9267 TIME SUBMITTED 03-SEP-2020 10:32AM
PAPER ID 62773224

Microb Ecol
DOI 10.1007/s00248-015-0620-9

FUNGAL MICROBIOLOGY

Diversity of Fungi on Decomposing Leaf Litter in a Sugarcane Plantation and Their Response to Tillage Practice and Bagasse Mulching: Implications for Management Effects on Litter Decomposition

Toshiko Miura[1] · Ainin Niswati[2] · I. G. Swibawa[3] · Sri Haryani[4] · Heru Gunito[4] · Satoshi Shimano[5] · Koichi Fujie[1] · Nobuhiro Kaneko[1]

Received: 7 July 2014 / Accepted: 20 April 2015

Abstract To minimize the degradation of soil organic matter (SOM) content in conventional sugarcane cropping, it is important to understand how the fungal community contributes to SOM dynamics during the decomposition of sugarcane leaf litter. However, our knowledge of fungal diversity in tropical agroecosystems is currently limited. Thus, we determined the fungal community structure on decomposing sugarcane leaf litter and their response to different soil management systems using the internal transcribed spacer region 1 (ITS1) amplicon sequencing method afforded by Ion Torrent Personal Genome Machine (PGM). The results indicate that no-tillage had positive effects on the relative abundance of Zygomycota and of some taxa that may prefer a moist environment over conventional tillage, whereas bagasse mulching decreased the richness of operational taxonomic units (OTUs) and had positive effect on the relative abundance of slow-growing taxa, which may prefer poor nutrient substrates. Furthermore, a combination of no-tillage and bagasse mulching increased the abundance of unique OTUs. We suggest that the alteration of fungal communities through the changes in soil management practices produces an effect on litter decomposition.

Keywords Fungi · Internal transcribed spacer region · Next-generation sequencing · Residue management · Nutrient cycling

Electronic supplementary material The online version of this article (doi:10.1007/s00248-015-0620-9) contains supplementary material, which is available to authorized users.

✉ Toshiko Miura
toshikom@ynu.ac.jp

[1] Graduate School of Environment and Information Sciences, Yokohama National University, 79-7 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan

[2] Department of Soil Science, University of Lampung, Bandar Lampung, Indonesia

[3] Department of Plant Pest and Diseases, University of Lampung, Bandar Lampung, Indonesia

[4] Research and Development Division of PT Gunung Madu Plantations, Sumatra, Indonesia

[5] Environmental Education Center, Miyagi University of Education, Sendai, Japan

Introduction

Sugarcane is cultivated primarily in tropical and subtropical regions, and recently, growing areas have been extended because of an increase in the demand for food and biofuel [1]. It is estimated that the current global cultivated sugarcane area is 26.08 million ha [2]. However, the soil organic matter (SOM) content is decreasing under conventional sugarcane monocropping [3–6]. The implementation of conservation tillage practice has increased in the sugarcane plantations of Brazil and Australia [7, 8]. In general, practicing no-till agriculture helps to increase and maintain SOM content [9–11]. No-tillage prevents soil carbon (C) loss resulting from erosion after tilling [12] and increases the proportion of macroaggregates, which results in the accumulation of SOM within aggregate structure [13]. In addition, the application of sugarcane bagasse (sugarcane fibers from which the juice has been extracted) to the soil has been found to increase SOM content [14]. By-products with a high C/N ratio (such as sugarcane bagasse) are slowly decomposed and thus can help to reduce soil erosion by covering the soil surface [15].

Published online: 03 May 2015

While these management changes may affect SOM dynamics, predicting the effects of management change on the C and nutrient cycling requires a more complete understanding of the factors that regulate litter decomposition. Sugarcane leaf litter, which amounts to 6–8 t dry weight ha^{-1} $year^{-1}$ [16], is important source and regulator of C and nutrient to the soil. To supply the C and nutrient from litter to the soil and to immobilize nutrients from soil to litter, fungal communities play a key role in these processes during decomposition. While changes in fungal community structure and species diversity may affect the decomposition of organic matter [17], few studies have focused on the effect of agricultural management on fungal communities in decomposing litter.

A previous study has demonstrated using terminal restriction fragment length (T-RF) polymorphism (T-RFLP) analysis that no-tillage increases T-RF richness, and bagasse mulching inhibits a specific fungal T-RF [18]. In addition, bagasse mulching can suppress the decomposition rate of sugarcane leaf litter. These findings indicate that changes in fungal community structures affect litter decomposition. However, the determination of fungal species and their physiology as associated with soils under different management practices is necessary for further elucidating the relationships between fungal community structure and litter decomposition.

Recently developed high throughput next-generation sequencing (NGS) methods can provide hundreds of thousands to tens of millions of DNA sequences per run [19], enabling the revelation of deeper microbial community connections in environmental samples than in the conventional methods.

This study aimed to reveal the effects of different tillage and bagasse mulching practices on fungal diversity and community structure in decomposing sugarcane leaf litter and to determine the relationship between fungal community structure and litter decomposition rate.

Materials and Methods

Site Description

The field study was conducted at a sugarcane plantation (4° 40′ S, 105° 13′ E, altitude c.a. 45 m) in Sumatra, Indonesia, from September 2010 to January 2011. The experimental site was located within a large area (approximately 25,000 ha) of the plantation. The Köppen system classifies the climate of the research area as Am. The total precipitation amounts at this site during the dry season (May 2010–September 2010) and the wet season (October 2010–April 2011) were 854 and 2,097 mm, respectively. The average air temperatures during the dry and wet season were 28.3 and 29.5 °C, respectively. The main soil type of the experimental area is Alisol [20], and soil physical and chemical characteristics particular of each treatment plot are provided in [18]. We used a split-plot design with soil tillage as the main factor and bagasse mulch as a secondary factor. The treatments were no-tillage without mulch (NT), no-tillage with mulch (NTM), conventional tillage without mulch (CT), and conventional tillage with mulch (CTM) repeated across four replicate blocks. Each plot was 25 m×25 m with a 5-m buffer zone adjacent to the road. The conventional tillage treatment plots were plowed three times in July 2010. For the mulch treatments, 80 t (wet weight) per hectare of bagasse was spread on the soil surface from August 1 to August 5, 2010. Eighty tons (wet weight) per hectare of organic fertilizer, consisting of five parts bagasse, three parts Blotong (filter cake), and three parts bagasse ash, were spread prior to plowing in the CT and CTM treatments and after planting in the NT and the NTM treatment. Inorganic fertilizers (N/P/K 120:80:180 kg/ha) were applied in all treatments at the time of planting. Sugarcane seed stems were planted during July 21–30, 2010. Herbicides were not applied to any of the treatments.

Litterbag Collection

Nylon bags (2-mm mesh size) of 20 cm×20 cm containing 10 g (dry weight) of brown sugarcane leaves cut into 15-cm length were placed on the soil surface on September 24, 2010. In the mulch treatments, the bags were covered with bagasse. Two litterbags per plot were collected after 124 days (January 25, 2011): one bag was used to measure mass loss, and one bag was used for molecular analysis of the fungal community. The leaf litter was sorted to remove roots and soil. The litter samples for mass loss were dried at 80 °C for 24 h. The ash-free dry mass was determined after grinding each sample in a grinding mill and then ashing one subsample (0.5 g) at 450 °C for 4 h in a muffle furnace. To prepare the samples for phospholipid fatty acid (PLFA) and molecular analysis, soil and loose roots were shaken off and the remaining roots on soil was removed with a sterilized spatula; then, the leaves were freeze-dried and stored at −20 °C until analysis. The protocol and the results of litter mass loss rate and PLFA were the same data as presented in the previous work [18], but the data of the fifth replicated plot of each treatment was omitted in this study to reduce the cost of DNA sequencing.

Analysis of Fungal Biomass in Sugarcane Leaf Litter

PLFA analysis was used to assess the fungal biomass. PLFAs were extracted from 0.5 g finely ground freeze-dried samples using a procedure based on those of [21] and [22]. Briefly, lipids were extracted with a one-phase chloroform–methanol–phosphate buffer, and the PLFA fraction was separated using silicic acid columns (BOND ELUT LRC-SI; Varian, Palo Alto, CA, USA) before trans-esterification with mild alkali and final uptake in dichloromethane. Methyl nonadecanoate (19:0) was added to each sample as an internal standard. The

fatty acid methyl esters were separated on a gas chromatograph with a Sherlock Microbial Identification System (MIDI, Newark, DE, USA). The fatty acids 18:2ω6 were used to estimate saprophytic fungal biomass [23].

DNA Sequence Analysis of Fungal Community Structure in Sugarcane Leaf Litter

DNA was isolated from 0.15 g of finely ground freeze-dried samples using an ISOIL for Beads Beating Kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Extracted DNA was quantified and checked for purity at the ratio of spectrophotometric absorbance (A 260/280 nm) (Nanodrop, Thermo Fisher Scientific, USA) prior to storage at −20 °C.

The fungal primers ITS1-F_KYO1/ITS2_KYO1 [24] were used to amplify the ITS1 region of fungal ribosomal DNA (rDNA). This primer set was designed to improve coverage across diverse taxonomic groups of fungi compared with existing primers [24]. The PCR mixture (20 μl) was prepared by combining 1 μl of DNA template (50 ng), 0.5 μl of 10 pmol μl^{-1} primers, and 10 μl of Thunderbird SYBR qPCR Mix (TOYOBO, Osaka, Japan). Real-time PCR was run on a StepOnePlus real-time PCR System (Applied Biosystems, Houston, USA) to determine the optimal cycle number by reference to cycle threshold (Ct) values. The real-time PCR conditions used were 94 °C for 4 min, 40 cycles of 94 °C for 30 s (denaturation), 50 °C for 1 min (annealing), and 72 °C for 90 s (extension) followed by 72 °C for 10 min. Ct values were defined as the number of cycles required for normalized fluorescence to reach a manually set threshold of 20 % total fluorescence. The results of the Ct value for each individual sample are presented in Table S1. PCR amplification was run on a GeneAmp PCR System 9700 (Applied Biosystems) with the same reagents and same PCR conditions (except for PCR cycle number) as the real-time PCR. The PCR products were purified using the Agencourt AMPure XP Reagent (Beckman Coulter, Brea, CA, USA). The purified PCR amplicons were end-repaired using the Ion Plus Fragment Library Kit (Life Technologies Inc., Grand Island, NY, USA), following the manufacturer's protocol. The end-repaired amplicons were purified using the Agencourt AMPure XP Reagent. Sequencing adapters with the sample identification barcoding key were ligated using an Ion Xpress Fragment Library Kit, following the manufacturer's protocol. The adapter-ligated and nick-translated amplicons were purified using the Agencourt AMPure XP Reagent. The concentrations of the prepared libraries were determined by quantitative PCR using the Ion Library Quantitation Kit (Life Technologies Inc.). The amount of library required for template preparation was calculated using the template dilution factor calculation described in the protocol. Diluted libraries were pooled for library amplification using the Ion One Touch and ES systems (Life Technologies Inc.). Emulsion PCR to incorporate the library to the sequencing beads was performed using the Ion OneTouch instrument with an Ion OneTouch System Template 200 Kit (Life Technologies Inc.). Finally, the library sample was sequenced on an Ion Torrent Personal Genome Machine using an Ion 318 chip and the Ion PGM 200 sequencing Kit (Life Technologies Inc.), following the manufacturer's protocols. The raw sequence data (.fastq file) are available in the DNA Data Bank of Japan (DDBJ) under accession number DRA002156.

Data Analysis

The sequence data were analyzed using the Mothur pipeline (v. 1.32.1) following a modified standard operating procedure [25]. In brief, the data were subjected to quality control, whereby each sequence was screened for a match to the sequencing primer and thresholds for average Phred quality score ($Q \geq 20$), ambiguous bases (count=0), and homopolymers (length≤8). Sequences shorter than 100 bp after quality trimming were not considered. All potentially chimeric sequences were identified using Mothur-embedded UCHIME (chimera.uchime) [26] and removed. The sequence data were subsampled to 11,711 sequences per sample (the smallest sample size) to reduce bias associated with different numbers of reads in the different samples [27]. Unique sequences were pairwise aligned (Needleman-Wunsch; [28]) and the resultant distance matrix clustered into operational taxonomic units (OTUs) using the nearest neighbor algorithm at >97 % similarity. Singleton OTUs were removed because many singletons are likely artifacts [29]. Classification of the sequences was performed using the UNITE + International Nucleotide Sequence Databases (INSD: NCBI, EMBL, DDBJ) ITS reference database (ver. 6; released on December 8, 2013) with the BLASTn algorithm [30].

The diversity of fungal communities in each treatment was compared using multiple metrics for rarefaction, observed OTU richness, Good's coverage (complement of the ratio between local singleton OTUs and the total sequence count), Simpson diversity index (1/*D*), Shannon diversity index (*H*), and Smith and Wilson's evenness index (E_{var}) calculated in Mothur software package. We chose E_{var} from several evenness indices because this index is independent of species richness [31]. The data were tested for normality using Shapiro-Wilk test and revealed no violation of the normality assumption, except the data for OTU abundances, as shown below. The main and interaction effect of tillage and mulch was assessed by a split-plot two-way analysis of variance (ANOVA) in R [32].

Community similarities across the treatment were visualized using nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis metrics after removal of rare sequences that have a total count less than 10. Ordination plots

were created using the "metaMDS" function in the R vegan package, which incorporated a square root transformation and then a Wisconsin double standardization of OTU abundances. We then assessed the effects of tillage and mulching on fungal community structure by analyzing the Bray-Curtis metrics in permutational multivariate analyses of variance (PERMANOVA) [33]. These analyses were conducted using the "Adonis" function of the R vegan package [34], with 999 permutations. The OTUs were analyzed for responses to treatments (split-plot two-way ANOVA of square root transformed and Wisconsin double-standardized OTU frequencies).

To assess and visualize the relationship between fungal community structure and litter decomposition rate, we overlaid this variable on the NMDS plot. The fitted smooth surface on the ordination was calculated using generalized additive models (GAMs) with thin-plate splines (based on the "ordisurf" function in the vegan package; [34]).

Results

Decomposition Rate and Fungal Biomass of Sugarcane Leaf Litter

The mass loss rate of sugarcane leaf litter after 124 days was lowest in the CTM treatment, followed by the NTM, CT, and NT treatments (Table 1). ANOVA revealed that the litter mass loss rate was significantly affected by mulch treatment.

The concentration of fungal PLFA was 3.2-fold greater in CTM and 2.4-fold greater in NT than in the CT treatment (Table 1). ANOVA revealed a significant effect of mulch treatment. There was, however, a tillage × mulch interaction effect: the positive effect of mulch on fungal PLFA was pronounced only under CT treatment.

Diversity

We investigated the litter fungal response to different agricultural managements using an Ion Torrent PGM dataset of 1,150,336 sequences with an average read length of 163.5±7.0 (mean±standard deviation (SD)). After omitting 73 % of sequence data because of homopolymers exceeding eight nucleotides, low average quality scores (Q<20), or insufficient read length (<100 bp), we retained 316,314 high-quality sequences with an average read length of 184.7±14.5 (mean±SD). The average number of reads per sample after omitting low-quality sequences was 19,770±7,258 (mean±SD). The numbers of sequences for each individual sample are listed in Table S1.

Clustering at 97 % identity produced 9,210 unique OTUs across the four soil management practices, of which a high number (8,506) were singletons. The 8,506 singletons made up 2.7 % of the total number of reads. After all singletons had been discarded, 704 OTUs remained and were used in the further analyses. Rarefaction analyses indicated a decline in the slope of the gradient (Fig. 1), and Good's coverage was remarkably high across all samples (Table 1), indicating an excellent overall OTU coverage afforded by the deep sequencing. The observed OTU richness was negatively affected by mulch treatment. On the other hand, Good's coverage and evenness (E_{var}) were affected positively by bagasse mulch treatment. There were no significant differences in the diversity indices (Simpson and Shannon) among any of the treatments.

Table 1 Litter mass loss, fungal PLFA, fungal OTU richness, coverage, and diversity indices across the four agricultural treatments

Treatment	Proportion of litter mass loss (%)	Fungal PLFA (nmol g^{-1} dry mass)	Number of observed OTUs	Good's coverage (%)	Simpson (1/D)	Shannon (H)	Evenness (E var)
CT	59.99 (4.46)	107.8 (40.6)	174.3 (22.6)	99.53 (0.067)	6.71 (2.61)	2.60 (0.31)	0.49 (0.01)
CTM	41.74 (10.19)	350.0 (118.4)	137.8 (15.9)	99.62 (0.061)	6.33 (3.45)	2.39 (0.51)	0.51 (0.01)
NT	63.81 (10.57)	253.5 (72.6)	191.8 (19.8)	99.50 (0.070)	7.30 (5.66)	2.56 (0.67)	0.48 (0.02)
NTM	55.85 (13.72)	207.5 (92.7)	131.0 (19.8)	99.63 (0.041)	5.15 (3.55)	2.14 (0.65)	0.50 (0.02)
ANOVA F value							
Tillage (T)	ns	ns	ns	ns	ns	ns	ns
Mulch (M)	*	*	***	**	ns	ns	*
T × M	ns	**	ns	ns	ns	ns	ns

Values in the upper portion of the table represent means. The values in parentheses indicate standard deviations (n=4). OTU clustering=97 % according to Mothur without singletons. Asterisks in the ANOVA results indicate significant results

ns not significant, *CT* conventional tillage, *CTM* conventional tillage with bagasse mulch, *NT* no-tillage, *NTM* no-tillage with bagasse mulch

*p<0.05; **p<0.01; ***p<0.001

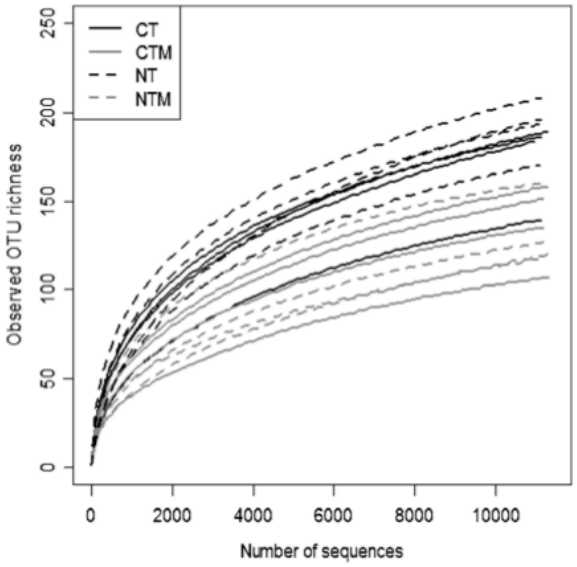

Fig. 1 Rarefaction curves of the observed fungal OTU richness for the litter samples at 97 % sequence similarity

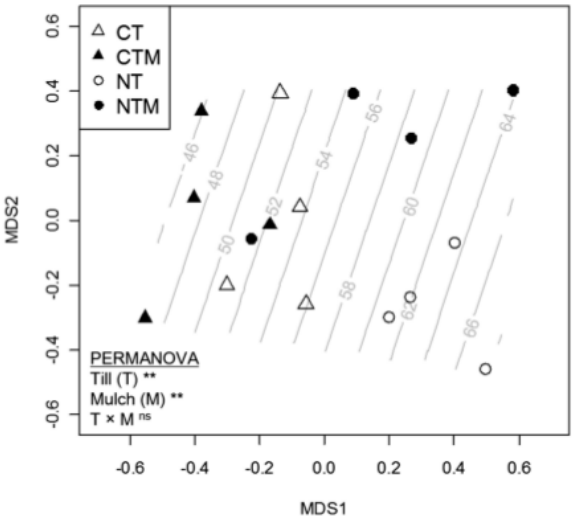

Fig. 2 The NMDS of litter fungal communities in the four treatments with GAM surface fits of litter decomposition rate (% mass loss). *Symbols* represent loading scores for the treatments. 2D stress=0.17. The relationship between litter decomposition and fungal community structure was significant in axis 1 ($p<0.05$). The result of the permutational multivariable analysis of variance (PERMANOVA) for differences in fungal community structure is also given (*$p<0.05$; **$p<0.01$; ***$p<0.001$; *ns*, not significant). *CT* conventional tillage; *CTM* conventional tillage with bagasse mulch; *NT* no-tillage; *NTM* no-tillage with bagasse mulch

Community Structure

Litter fungal communities at the phylum level across the four agricultural managements were dominated by Ascomycota (81.48 %) according to taxonomy assignment followed by Basidiomycota (17.94 %) and other fungal lineages (0.58 %), including Chytridiomycota, Glomeromycota, and Zygomycota (Table 2). The number of Zygomycota was significantly higher under no-tillage treatment. The other phyla did not statistically differ among treatments.

PERMANOVA results showed that the effect of tillage and bagasse mulching on fungal community structure was significant (Fig. 2). NMDS plots demonstrated that tillage and no-tillage treatments were clearly separated along axis 1, CT and CTM treatment were located on negative axis 1, whereas NT and NTM were located on positive axis 1 (Fig. 2). By contrast, the NMDS plots indicated that the effect of bagasse mulch treatment varied with tillage treatment. With the bagasse mulching under CT treatment, the points were shifted toward more negative axis 1. With the bagasse mulching under NT treatment, the points were shifted toward positive axis 2.

Table 2 Relative abundance of fungal phyla (% of the total sequences in each sample) across the four agricultural treatments

	Ascomycota	Basidiomycota	Zygomycota	Glomeromycota	Chytridiomycota	Unclassified fungi
CT	87.8 (14.7)	11.4 (13.8)	0.03 (0.05)	0.05 (0.04)	0.03 (0.04)	0.64 (0.97)
CTM	76.3 (24.7)	22.3 (24.2)	0.02 (0.02)	0.02 (0.03)	0.004 (0.009)	0.39 (0.54)
NT	90.6 (6.70)	8.37 (6.31)	0.19 (0.20)	0.16 (0.26)	0.012 (0.009)	0.67 (1.08)
NTM	78.6 (31.9)	21.0 (32.2)	0.14 (0.13)	0.05 (0.04)	0.007 (0.015)	0.20 (0.18)
ANOVA *F* value						
Tillage (T)	ns	ns	*	ns	ns	ns
Mulch (M)	ns	ns	ns	ns	ns	ns
T × M	ns	ns	ns	ns	ns	ns

Values in the upper portion of the table represent means. The values in parentheses indicate standard deviations ($n=4$). Asterisks in the ANOVA results indicate significant results

CT conventional tillage, *CTM* conventional tillage with bagasse mulch, *NT* no-tillage, *NTM* no-tillage with bagasse mulch, *ns* not significant

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 3 Taxonomic assignment of the top 19 most abundant OTUs (relative abundance of >1 % of the total sequences) in the litter samples, based on BLAST analysis against the UNITE+INSD database

OTU no.	Best BLASTn match (uncultured and environmental excluded)							Overall abundance (%)
	UNITE taxon name	Phylum	Family	Accession	*E* value	Identity (%)	Coverage (%)	
1	*Podospora* sp.	A	Lasiosphaeriaceae	JX984769	7E-95	94	100	23.31
2	*Resinicium saccharicola*	B	Meruliaceae	DQ826549	1E-127	100	100	12.85
3	*Dokmaia* sp. 1 TMS 2011	A	Incertae sedis	HQ631012	2E-57	99	100	8.88
4	*Arnium* sp.	A	Lasiosphaeriaceae	JX317197	2E-109	98	100	5.15
5	Ascomycota	A		GQ851818	2E-89	93	100	5.01
6	Sordariales	A		EU686925	1E-66	91	84	3.97
7	*Trichocladium pyriforme*	A	Chaetomiaceae	AM292048	2E-33	90	98	2.63
8	*Podospora* sp.	A	Lasiosphaeriaceae	JX984791	1E-56	89	100	2.43
9	*Volutella* sp.	A	Nectriaceae	HM136667	2E-33	88	98	2.05
10	Sordariomycetes	A		AJ875391	2E-55	84	100	1.87
11	Ascomycota.	A		GQ851818	2E-46	83	100	1.82
12	*Trichocladium pyriforme*	A	Chaetomiaceae	AM292048	3E-82	96	100	1.79
13	Ascomycota	A		GQ851818	2E-33	87	89	1.75
14	*Chaetomium* sp. ATT182	A	Chaetomiaceae	HQ607879	2E-33	92	85	1.69
15	*Apodus* sp.	A	Lasiosphaeriaceae	JF519313	3E-78	88	100	1.63
16	*Phellinidium sulphurascens*	B	Hymenochaetaceae	KF996528	5E-18	96	50	1.46
17	*Cercophora* sp. TMS 2011	A	Lasiosphaeriaceae	HQ631039	6E-66	86	100	1.30
18	Fungal sp. ARIZ L177	A	Chaetomiaceae	FJ612719	3E-36	93	85	1.25
19	*Pleosporales* sp. 1 TMS 2011	A		HQ631002	3E-21	100	49	1.01

A Ascomycota, *B* Basidiomycota, *CT* conventional tillage, *CTM* conventional tillage with bagasse mulch, *NT* no-tillage, *NTM* no-tillage with bagasse mulch

Among the 704 OTUs (261 OTUs without less than 10 rare sequences), 19 OTUs each accounted for >1 % of the total number of sequences (Table 3). The three most abundant OTUs were putatively *Podospora* sp. (23 % of the sequences), *Resinicium saccharicola* (13 % of the sequences), and *Dokmaia* sp. 1 TMS 2011 (9 % of the sequences). The relative abundance of these OTUs did not differ among the treatments, according to the ANOVA results. On the other hand, the 4th (putatively *Arnium* sp.), the 11th (putatively Ascomycota), and 18th (putatively fungal sp. ARIZ L177) most abundant OTUs were affected significantly by tillage (Table 4). NMDS ordination of OTUs indicated that the relative abundance of these OTUs was higher in tillage treatments than in no-tillage treatments. In addition, the 5th (putatively uncultured Hypocreales) and 13th (putatively *Phaeoisaria clematidis*) OTUs were significantly affected by bagasse mulch treatment. OTU ordinations of NMDS axis 2 indicated that these OTUs were more abundant in the bagasse mulch treatment than in the no-mulch treatment. All OTUs that were significantly affected by the treatments are presented in Table 3. These findings showed that certain OTUs were increased by the no-tillage treatment in low-abundant OTUs, at less than 1 % of relative abundance (e.g., OTU32, putatively *Paraphaeosphaeria* sp. CBS 97895; OTU35, putatively Sordariales). In addition, certain OTUs were decreased by bagasse mulch in low-abundant OTUs (e.g., OTU102, putatively uncultured Ascomycota; OTU209, putatively *Mortierella* sp.). Furthermore, some OTUs were affected by tillage × mulch interaction. For example, OTU23 (putatively *Sporobolomyces gracilis*) was increased by bagasse mulch, but the effect of bagasse mulch on this OTU only occurred when there was no-tillage treatment.

NMDS ordination of the fungal community structure revealed a significant and linear relationship with the litter mass loss rate (Fig. 2). The litter mass loss rate was positively and significantly correlated with NMDS axis 1 ($p<0.05$). Fungal communities of the CTM treatment are located on the negative NMDS axis 1, and they occur at sites with the lowest average litter mass loss rate. Inversely, fungal communities of the NT plot are located on the positive NMDS axis 1, and they occur at the highest average litter mass loss rate.

Discussion

The result of our deep amplicon sequence analysis indicated that bagasse mulching decreased fungal OTU richness, whereas the tillage treatment had no effect. However, a previous T-

Table 4 Results of the two-way ANOVA testing the effects of tillage, bagasse mulching, and their interaction on the relative abundance of the fungal OTUs

OTU No.	Best BLASTn match (uncultured and environmental excluded)					Split-plot two-way ANOVA		
	UNITE taxon name	Accession	*E* value	Identity (%)/ coverage (%)	Source	Till (T)	Mulch (M)	T × M
Positive effect of no-tillage								
32	*Paraphaeosphaeria* sp. CBS 97895	JX496120	1E-65	100/97	Soil, Papua (Verkley et al. [47])	*		
35	Sordariales	AB255295	2E-85	96/88	Leaf of bamboo (Sasa), Osaka, Japan (Morakotkarn et al. [48])	**		
44	*Onnia tomentosa*	AF237729	5E-23	100/53	Root of *Picea glauca*, B.C., Canada (Germain et al. [49])	*		
48	Uncultured glomus	KF849676	3E-20	90/65	Root of maize, Guangzhou, China (Zeng et al. [50])	*		
58	*Plectosphaerella* sp.	JF340251	9E-56	98/100	Healthy stem of *Alnus glutinosa*, Latvia (Arhipova et al. 2012)	**		
173	*Crustoderma corneum*	KC585319	8E-20	100/22	Brown-rot of *Pinus* species, Småland, Sweden (Nakasone [52])	*		
186	*Acremonium* sp.	HM162159	3E-108	99/100	Grass roots, Texas, USA (Kivlin and Hawkes [53])	*		
194	*Candida blankii*	KF963314	1E-18	97/21	Unpublished	**		
233	*Lasiosphaeria glabrata*	AY587914	2E-28	86/100	Dead deciduous trees, Denmark (Miller and Huhndorf [54])	*		
Negative effect of no-tillage								
4	*Arnium* sp.	JX317197	2E-109	98/100	Orchid rhizosphere, Minas Gerais, Brazil (Oliveira et al. [55])	*		
11	Ascomycota	GQ851818	2E-46	83/100	Unpublished	*		
18	Fungal sp. ARIZ L177 (Chaetomiaceae)	FJ612719	3E-36	93/85	Seed of a neotropical pioneer tree (U'ren et al. [56])	*		
43	*Trichoderma harzianum*	HG940473	2E-28	95/59	Unpublished	*		
52	Uncultured Ascomycota (Sordariomycetes)	AB769884	1E-42	82/100	Unpublished	*		
66	*Stylonectria* sp.	HQ607980	8E-26	88/100	Nest of attine ants, Central Texas, USA (Rodrigues et al. [57])	*		
79	*Helicoma isiola*	EF010926	6E-23	89/71	Unpublished	*		
83	*Periconia* sp.	GQ999275	2E-52	95/100	Unpublished	**		
99	Uncultured fungus (Cephalothecaceae)	JQ081418	2E-45	91/71	Nest soils of leaf-cutting ants, Brazil (Rodrigues et al. [58])	**		
106	*Microascus brevicaulis*	KC461533	2E-15	98/23	Vermicompost (Grantina-Ievina et al. [59])	*		
143	*Stachybotrys* cf *elegans*	KC305357	1E-44	96/97	Unpublished	*		
154	Fungal endophyte	KF435461	3E-22	100/35	Leaves in tropical forest, Panama (Higginbotham et al. [60])	*		
169	*Sebacinales* group B sp.	JX317483	3E-74	88/100	Orchid rhizosphere, Minas Gerais, Brazil (Oliveira et al. [55])	*		
171	Uncultured fungus (Sordariomycetes)	AJ875386	1E-77	90/100	*Phragmites australis*, Mainau, Germany (Neubert et al. [61])	*		

Table 4 (continued)

OTU No.	Best BLASTn match (uncultured and environmental excluded)					Split-plot two-way ANOVA		
	UNITE taxon name	Accession	*E* value	Identity (%)/ coverage (%)	Source	Till (T)	Mulch (M)	T × M
Positive effect of mulch								
5	Uncultured Hypocreales	GQ924027	2E-84	95/88	*Bouteloua gracilis*, New Mexico, USA (Herrera et al. [62])	*		
13	*Phaeoisaria clematidis*	EU552148	2E-24	92/57	Proteaceae, Cape, South Africa (Marincowitz et al. [63])	**		
30	*Pyrigemmula aurantiaca*	HM241692	5E-23	92/73	Bark of living wood, Hungary (Magyar et al. [64])	*		
64	*Sebacinaceae* sp. 9 MB_2012	JX138552	6E-45	91/66	Orchid roots, Western Australia (Sommer et al. [65])	*		
88	*Apodus oryzae*	AY681200	1E-45	89/98	? (Cai et al. [66])	*		
247	Uncultured fungus (Strophariaceae)	JX981801	9E-19	95/29	Unpublished	**		
Negative effect of mulch								
50	Uncultured Pezizales	FJ552762	8E-21	100/53	Forest soil, British Columbia, Canada (Hartmann et al. [67])		*	
81	*Microdiplodia* sp.	EU686121	3E-43	82/100	*Bazzania tayloriana*, New Zealand (Davis and Shaw [68])		*	
102	Uncultured Ascomycota (Tubeufiaceae)	EU490000	8E-41	95/100	Soils in mixed grass prairie, Texas, USA (Hollister et al. [69])		*	
114	Uncultured fungus (Tremellales)	GU998743	1E-17	96/28	Unpublished		*	
115	*Myrothecium* sp.	EU750694	1E-54	94/100	Unpublished		*	
153	*Edenia* sp.	JF737856	1E-48	98/96	Unpublished		*	
209	*Mortierella* sp.	KC191742	5E-48	99/100	Unpublished		*	
Interaction effect of tillage × mulch								
23	*Sporobolomyces foliicola*	JX401377	7E-70	88/100	Unpublished		*	*
36	Uncultured Ascomycota (Chaetothyriales)	HM239943	7E-22	85/87	Unpublished			*
49	Uncultured Ascomycota (Hypocreales)	EU372830	5E-111	97/100	Unpublished			*
54	*Tricharina groenlandica*	JQ824125	2E-19	95/37	Greenland (Stielow et al. [70])			**
62	*Fomes* sp.	KF541331	2E-19	98/34	Unpublished	**	**	**
133	Uncultured fungus (Pyronemataceae)	FN397181	2E-21	98/23	Truffle grounds, Le Montat, France (Napoli et al. [71])	*	**	*
146	*Bullera* sp.	AY313025	8E-99	99/100	Unpublished	*	*	*
165	Uncultured *Tomentella*	EU668215	4E-21	100/31	Orchid in Europe (Bidartondo and Read [72])	*	**	*
174	*Ophiocordyceps sphecocephala*	GU723779	5E-28	96/75	*Camponotus gigas*, Thailand (Luangsa-Ard et al. [73])			*
179	*Phaeoacremonium* sp.	JN890453	1E-53	85/85	Soils in tropical rain forest, Guyana (McGuire et al. [74])			*

Table 4 (continued)

OTU No.	Best BLASTn match (uncultured and environmental excluded)					Split-plot two-way ANOVA		
	UNITE taxon name	Accession	*E* value	Identity (%)/ coverage (%)	Source	Till (T)	Mulch (M)	T × M
197	*Leptosphaerulina chartarum*	KJ398148	4E-103	93/100	*Eurya nitida* in dry tropical forests, Ghats, India (Rajulu et al. [75])			*
216	*Rhizophagus* sp.	AJ567763	2E-60	88/100	Soils in mountain meadows, Thuringia, Germany (Börstler et al. [76])	*	*	*
257	*Cryptococcus* sp.	JQ666366	7E-20	100/25	Unpublished			*

Asterisks in the ANOVA results indicate significant results. Judgment as to whether the effects were positive or negative was derived from the NMDS scores. Taxonomic assignment of each OTU was based on BLAST analysis against the UNITE+INSD database. Source indicates the source of the sequence associated with the accession. Unpublished or unclear sources are indicated "unpublished" or "?", respectively. The references of the sources were listed in [47–76]

CT conventional tillage, *CTM* conventional tillage with bagasse mulch, *NT* no-tillage, *NTM* no-tillage with bagasse mulch

*$p<0.05$; **$p<0.01$; ***$p<0.001$

RFLP study showed the opposite result: no-tillage treatment increased fungal T-RF richness [18]. The T-RFLP can access only the top 100 most abundant T-RFs of the target community [35, 36], and a single T-RF peak can often represent multiple species [37]. On the other hand, the NGS technique can provide much information regarding the total members of the community at the sequence level. Therefore, the T-RFLP profile represents the diversity of dominant species, whereas our deep amplicon sequencing could obtain the community profile, including low-abundant taxa. Nevertheless, the fact that different reverse primers were used in both approaches might have introduced further distinctions in the recovery of community structure cannot be excluded.

The slightly higher evenness (E_{var}) in bagasse mulch treatments than in no-mulch treatments is likely to be correlated with Good's coverage. According to the result of Good's coverage, the bagasse mulch treatments had a lower number of singletons than no-mulch treatments had. Therefore, the higher evenness in bagasse mulch treatments might reflect the lower number of rare species in bagasse mulch treatments than in no-mulch treatments.

Although the fungal community structure exhibited significant differences among treatments, the relative abundance of the three most dominant OTUs was not affected by treatment. The functions of *Podospora* spp., which was the most dominant fungal OTU in the sugarcane leaf litter, are not known. However, the same genus, *Podospora anserina*, has the ability to degrade cellulose [38]. The functions of *Resinisium saccharicola* related to organic matter degradation are also unknown. However, *Recinisium bicolor*, which were closely related to *R. saccharicola*, were famous as white-rot fungi [39]. *Dokmaia* spp. have been detected from strains that were isolated from culture medium of decomposing sugarcane tissues [40]. The same genus of *Dokmaia monthadangii* is known as saprophytes [41]. Consequently, these fungal taxa are considered to play an important role in litter decomposition. However, although the relative abundance of these three most dominant taxa was not affected by any treatments, the litter decomposition rate was significantly affected by bagasse mulch treatment. In addition, there was a significant correlation between fungal community structure and litter decomposition rate. Therefore, changes in the lower abundant taxa responding to the agricultural managements may correspond to the decomposition rate. In the OTUs which were positively affected by bagasse mulch treatment, many putative species of these OTUs have been discovered from infertile environments or nutrient-poor substrates such as *Bouteloua gracilis* (slow-growing and high drought tolerance) in New Mexico, fynbos in South Africa, an old and weathered land in Australia and the bark of a tree (Table 4). Bagasse has lower N content (0.3 % N dry mass) than the sugarcane leaf litter (0.6 % N dry mass) and has high lignin content [42]; thus, it can be considered that the poor N and high lignin content of bagasse is not favorable for many species of fungi but promotes the

growth of specific fungal taxa that can utilize N-poor and lignin-rich substrates. In a high N environment, lignin decomposition is limited as slow-growing lignin decomposing fungi are unable to compete with fast-growing microbes and are eliminated from the decomposer community [43]. According to this ecological argument, bagasse should promote slow-growing fungal decomposers, which may reflect the slow decomposition rate of sugarcane leaf litter under bagasse mulch treatment. The fungal species that increased under bagasse mulch treatment might play a major role in the degradation of organic matter containing high lignin and row nutrients. Therefore, understanding the functions of these species might contribute to the effective use of persistent agricultural wastes.

Although there was no significant difference in litter mass loss rate between the CT and NT treatment, the fungal community structure was significantly affected by tillage treatment. *Plectosphaerella* sp., which is the putative species of OTU58 that was increased by no-tillage treatment, has been known to have the ability to degrade (hemi)cellulose [44]. The putative species of OTUs that were positively affected by no-tillage treatment have been discovered from cool and wet environments such as freshwater, the Japanese bamboo forest, the North American coniferous forest, Latvia alder, and Denmark (Table 4). *Parapaheispheria* of the same genus as the best hit species of OTU32 has been suggested to prefer moist environments [45]. On the other hand, the best hit species of some OTUs that were affected negatively by no-tillage treatment were derived from tropical and subtropical forests of Central and South America and China (Table 4). Litter under no-tillage treatment had higher moisture content than CT treatment did [18], and the soil moisture content under no-tillage treatment was more stable than under CT treatment (data not shown). Therefore, it can be considered that species that prefer humid and stable climate conditions were increased under the no-tillage treatment. In addition, the phylum level compositions showed that the no-tillage treatment yielded a higher Zygomycota abundance than the conventional tillage treatment did. Zygomycota are known to be fast-growing fungi capable of rapidly exploring new substrates and primarily utilizing simple soluble substrates [46]. As previously discussed, higher weed biomass and species in the NT treatment may create the physical and chemical heterogeneity in the no-till litter [18]. Thus, although the biomass of weeds was smaller than sugarcane, the addition of different quality litter to the soil surface could increase the resource heterogeneity for fungi. Therefore, the litter decomposition process may differ between the two tillage systems.

In addition, when bagasse mulch was combined with no-tillage treatment, some particular OTUs were greater than when the bagasse mulch was combined with conventional tillage treatment (Table 4). The occurrence number of OTU 23 (putatively *S. gracilis*) was significantly higher in the NTM than in the CTM treatment (Tukey's HSD test: $p=0.047$). According to the PLFA analysis, the fungal biomass also differed between the CTM and NTM treatment (Table 1). Therefore, different tillage practices alter the fungal community structure responding to bagasse mulch.

Conclusion

Our findings revealed that the application of conservation tillage, bagasse mulching, and a combination of the two agricultural managements practices in a sugarcane plantation produced differences in fungal diversity and community structure during litter decomposition. Compared with conventional tillage management, no-tillage management tended to increase the abundance of fungal taxa that are fast-growing and may prefer moist and cool environments, whereas bagasse mulching tended to increase the abundance of fungal taxa that are slow-growing and may prefer nutrient-poor environments. We suggest that the alteration of fungal communities through changes in agricultural managements will produce a significant effect on litter decomposition rates and processes. Therefore, capturing the comprehensive behavior of the fungal community in these agroecosystems may contribute to accurately predicting the effect of agricultural managements on biochemical processes in soils. However, the roles of many of the fungal taxa identified in this study are yet to be understood.

Acknowledgments Our study was supported in part by a Grant-in-Aid for the Global COE Program E03 for Global Eco-Risk Management from Asian Viewpoints; the YNU International Environmental Leaders Program in Sustainable Living with Environmental Risk funded by Promoting Science and Technology System Reform of the Promotion of Science and Technology, Japan; and a grant from Yokohama National University and MEXT KAKENHI Grant Number 25220104. We thank the staff at the Gunung Madu Plantations for permitting this study and supporting the field experiment, Prof. John Hendri and Prof. Andi Setiawan in the Department of Chemistry FMIPA at the University of Lampung, and Dr. Udin Hasanuddin in the Department of Agroindustrial Technology, Faculty of Agriculture at the University of Lampung for supporting the field laboratory experiments. We are also grateful to Dr. T. Nakamori at the Graduate School of Environment and Information Sciences, Yokohama National University, for helpful conversations regarding sequencing analysis. The data parsing processes were performed using the super computer system provided by the National Institute of Genetics (NIG), Japan.

References

1. FAO. (2008). *Biofuels: prospects, risks and opportunities*. FAO Agriculture Series (Vol. 39.). Rome: Food and Agriculture Organization of the United Nations (FAO)
2. FAO (2014) FAOSTAT online statistical service. Food and Agriculture Organization of the United Nations (FAO), Rome
3. Bonde TA, Christensen BT, Cerri CC (1992) Dynamics of soil organic matter as reflected by natural 13C abundance in particle size fractions of forested and cultivated oxisols. Soil Biol Biochem 24(3):275–277. doi:10.1016/0038-0717(92)90230-U

4. Hartemink AE (1998) Soil chemical and physical properties as indicators of sustainable land management under sugar cane in Papua New Guinea. Geoderma 85(4):283–306. doi:10.1016/S0016-7061(98)00048-2
5. Dominy C, Haynes R (2002) Influence of agricultural land management on organic matter content, microbial activity and aggregate stability in the profiles of two Oxisols. Biol Fertil Soils 36(4):298–305. doi:10.1007/s00374-002-0542-9
6. Osher LJ, Matson PA, Amundson R (2003) Effect of land use change on soil carbon in Hawaii. Biogeochemistry 65(2):213–232. doi:10.1023/A:1026048612540
7. Galdos MV, Cerri CC, Cerri CEP (2009) Soil carbon stocks under burned and unburned sugarcane in Brazil. Geoderma 153(3–4):347–352. doi:10.1016/j.geoderma.2009.08.025
8. Stirling GR, Moody PW, Stirling AM (2010) The impact of an improved sugarcane farming system on chemical, biochemical and biological properties associated with soil health. Appl Soil Ecol 46(3):470–477. doi:10.1016/j.apsoil.2010.08.015
9. Machado PLOA, Silva CA (2001) Soil management under no-tillage systems in the tropics with special reference to Brazil. Nutr Cycl Agroecosyst 61(1–2):119–130. doi:10.1023/A:1013331805519
10. Jiménez JJ, Lal R (2006) Mechanisms of C sequestration in soils of Latin America. Crit Rev Plant Sci 25(4):337–365. doi:10.1080/07352680600942400
11. Neto MS, Scopel E, Corbeels M, Cardoso AN, Douzet J-M, Feller C, Bernoux M (2010) Soil carbon stocks under no-tillage mulch-based cropping systems in the Brazilian Cerrado: an on-farm synchronic assessment. Soil Tillage Res 110(1):187–195. doi:10.1016/j.still.2010.07.010
12. Conant RT, Easter M, Paustian K, Swan A, Williams S (2007) Impacts of periodic tillage on soil C stocks: a synthesis. Soil Tillage Res 95(1–2):1–10. doi:10.1016/j.still.2006.12.006
13. Six J, Feller C, Denef K, Ogle SM, de Moraes JC, Albrecht A (2002) Soil organic matter, biota and aggregation in temperate and tropical soils—effects of no-tillage. Agronomie 22(7–8):755–775. doi:10.1051/agro:2002043
14. Taja H, Vanderzaag P (1991) Organic residue management in the hot tropics: influence on the growth and yield of Solanum potato and maize. Trop Agric 68(2):111–118
15. Scopel E, Findeling A, Guerra EC, Corbeels M (2005) Impact of direct sowing mulch-based cropping systems on soil carbon, soil erosion and maize yield. Agron Sustain Dev 25(4):425–432. doi:10.1051/agro:2005041
16. Singh P, Suman A, Tiwari P, Arya N, Gaur A, Shrivastava AK (2007) Biological pretreatment of sugarcane trash for its conversion to fermentable sugars. World J Microbiol Biotechnol 24(5):667–673. doi:10.1007/s11274-007-9522-4
17. Deacon LJ, Janie Pryce-Miller E, Frankland JC, Bainbridge BW, Moore PD, Robinson CH (2006) Diversity and function of decomposer fungi from a grassland soil. Soil Biol Biochem 38(1):7–20. doi:10.1016/j.soilbio.2005.04.013
18. Miura T, Niswati A, Swibawa IG, Haryani S, Gunito H, Kaneko N (2013) No tillage and bagasse mulching alter fungal biomass and community structure during decomposition of sugarcane leaf litter in Lampung Province, Sumatra, Indonesia. Soil Biol Biochem 58:27–35. doi:10.1016/j.soilbio.2012.10.042
19. Metzker ML (2010) Sequencing technologies—the next generation. Nature reviews. Genetics 11(1):31–46. doi:10.1038/nrg2626
20. Driessen P, Deckers J, Spaargaren O, Nachtergaele F (2001) In world soil resources reports 2000. Food and Agriculture Organization (FAO), Rome, Lecture notes on the major soils of the world
21. Frostegård Å, Tunlid A, Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbiol Methods 14(3):151–163. doi:10.1016/0167-7012(91)90018-L
22. Niwa S, Kaneko N, Okada H, Sakamoto K (2008) Effects of fine-scale simulation of deer browsing on soil micro-foodweb structure and N mineralization rate in a temperate forest. Soil Biol Biochem 40(3):699–708. doi:10.1016/j.soilbio.2007.10.004
23. Frostegård Å, Tunlid A, Bååth E (2011) Use and misuse of PLFA measurements in soils. Soil Biol Biochem 43(8):1621–1625. doi:10.1016/j.soilbio.2010.11.021
24. Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS ONE 7(7), e40863. doi:10.1371/journal.pone.0040863
25. Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS ONE 6(12), e27310. doi:10.1371/journal.pone.0027310
26. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. doi:10.1093/bioinformatics/btr381
27. Gihring TM, Green SJ, Schadt CW (2012) Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. Environ Microbiol 14(2):285–290. doi:10.1111/j.1462-2920.2011.02550.x
28. Needleman SB, Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. J Mol Biol 48(3):443–453. doi:10.1016/0022-2836(70)90057-4
29. Tedersoo L, Nilsson RH, Abarenkov K, Jairus T, Sadam A, Saar I, Kõljalg U (2010) 454 pyrosequencing and Sanger sequencing of tropical mycorrhizal fungi provide similar results but reveal substantial methodological biases. New Phytol 188(1):291–301. doi:10.1111/j.1469-8137.2010.03373.x
30. Abarenkov K, Henrik Nilsson R, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Kõljalg U (2010) The UNITE database for molecular identification of fungi—recent updates and future perspectives. New Phytol 186(2):281–285. doi:10.1111/j.1469-8137.2009.03160.x
31. Smith B, Wilson JB (1996) A consumer's guide to evenness indices. Oikos 76(1):70. doi:10.2307/3545749
32. R Development Core Team (2009) A language and environment for statistical computing
33. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26(1):32–46. doi:10.1111/j.1442-9993.2001.01070.pp.x
34. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB (2013) Helene Wagner. Vegan: Community Ecology Package
35. Dickie IA, FitzJohn RG (2007) Using terminal restriction fragment length polymorphism (T-RFLP) to identify mycorrhizal fungi: a methods review. Mycorrhiza 17(4):259–270. doi:10.1007/s00572-007-0129-2
36. Van Elsas JD, Boersma FGH (2011) A review of molecular methods to study the microbiota of soil and the mycosphere. Eur J Soil Biol 47(2):77–87. doi:10.1016/j.ejsobi.2010.11.010
37. Anderson IC, Cairney JWG (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ Microbiol 6(8):769–779. doi:10.1111/j.1462-2920.2004.00675.x
38. Žifčáková L, Baldrian P (2012) Fungal polysaccharide monooxygenases: new players in the decomposition of cellulose. Fungal Ecol 5(5):481–489. doi:10.1016/j.funeco.2012.05.001
39. Nakasone KK (2007) Morphological and molecular studies on Resinicium s. str. Can J Bot 85(4):420–436. doi:10.1139/B07-035
40. Shrestha P, Szaro TM, Bruns TD, Taylor JW (2011) Systematic search for cultivatable fungi that best deconstruct cell walls of

Miscanthus and sugarcane in the field. Appl Environ Microbiol 77(15):5490–5504. doi:10.1128/AEM.02996-10
41. Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R (2007) A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. Microb Ecol 53(4): 579–590. doi:10.1007/s00248-006-9117-x
42. Chandel AK, da Silva SS, Carvalho W, Singh OV (2012) Sugarcane bagasse and leaves: foreseeable biomass of biofuel and bio-products. J Chem Technol Biotechnol 87(1):11–20. doi:10.1002/jctb.2742
43. Coûteaux M-M, Mousseau M, Célérier M-L, Bottner P, Couteaux M-M, Celerier M-L (1991) Increased atmospheric CO2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. Oikos 61(1):54. doi:10.2307/3545406
44. Jiménez DJ, Korenblum E, van Elsas JD (2014) Novel multispecies microbial consortia involved in lignocellulose and 5-hydroxymethylfurfural bioconversion. Appl Microbiol Biotechnol 98(6):2789–2803. doi:10.1007/s00253-013-5253-7
45. Herrera J, Poudel R, Nebel KA, Collins SL (2011) Precipitation increases the abundance of some groups of root-associated fungal endophytes in a semiarid grassland. Ecosphere 2(4):1–14. doi:10.1890/ES11-00001.1
46. Kjøller A, Struwe S (2002) Fungal communities, succession, enzymes, and decomposition. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications, vol 4. Marcel Dekker, New York, pp 267–284
47. Verkley GJM, Dukik K, Renfurm R, Göker M, Stielow JB (2014) Novel genera and species of coniothyrium-like fungi in Montagnulaceae (Ascomycota). Persoonia 32:25–51. doi:10.3767/003158514X679191
48. Morakotkarn D, Kawasaki H, Seki T (2007) Molecular diversity of bamboo-associated fungi isolated from Japan. FEMS Microbiol Lett 266(1):10–19. doi:10.1111/j.1574-6968.2006.00489.x
49. Germain H, Laflamme G, Bernier L, Boulet B, Hamelin RC (2002) DNA polymorphism and molecular diagnosis in Inonotus spp. Can J Plant Pathol 24(2):194–199. doi:10.1080/07060660309506995
50. Zeng H, Tan F, Zhang Y, Feng Y, Shu Y, Wang J (2014) Effects of cultivation and return of Bacillus thuringiensis (Bt) maize on the diversity of the arbuscular mycorrhizal community in soils and roots of subsequently cultivated conventional maize. Soil Biol Biochem 75:254–263. doi:10.1016/j.soilbio.2014.04.024
51. Arhipova N, Gaitnieks T, Donis J, Stenlid J, Vasaitis R (2012) Heart-rot and associated fungi in Alnus glutinosa stands in Latvia. Scand J For Res 27(4):327–336. doi:10.1080/02827581.2012.670727
52. Nakasone KK (1984) Taxonomy of Crustoderma (Aphyllophorales, Corticiaceae). Mycologia 76(1):40. doi:10.2307/3792834
53. Kivlin SN, Hawkes CV (2011) Differentiating between effects of invasion and diversity: impacts of aboveground plant communities on belowground fungal communities. New Phytol 189(2):526–535. doi:10.1111/j.1469-8137.2010.03494.x
54. Miller AN, Huhndorf SM (2004) Using phylogenetic species recognition to delimit species boundaries within Lasiosphaeria. Mycologia 96(5):1106–1127
55. Oliveira SF, Bocayuva MF, Veloso TGR, Bazzolli DMS, da Silva CC, Pereira OL, Kasuya MCM (2014) Endophytic and mycorrhizal fungi associated with roots of endangered native orchids from the Atlantic Forest, Brazil. Mycorrhiza 24(1):55–64. doi:10.1007/s00572-013-0512-0
56. U'ren JM, Dalling JW, Gallery RE, Maddison DR, Davis EC, Gibson CM, Arnold AE (2009) Diversity and evolutionary origins of fungi associated with seeds of a neotropical pioneer tree: a case study for analysing fungal environmental samples. Mycol Res 113(4):432–449. doi:10.1016/j.mycres.2008.11.015
57. Rodrigues A, Mueller UG, Ishak HD, Bacci M, Pagnocca FC (2011) Ecology of microfungal communities in gardens of fungus-growing ants (Hymenoptera: Formicidae): a year-long survey of three species of attine ants in Central Texas. FEMS Microbiol Ecol 78(2):244–255. doi:10.1111/j.1574-6941.2011.01152.x
58. Rodrigues A, Passarini MRZ, Ferro M, Nagamoto NS, Forti LC, Bacci M, Pagnocca FC (2014) Fungal communities in the garden chamber soils of leaf-cutting ants. J Basic Microbiol 54(11):1186–1196. doi:10.1002/jobm.201200458
59. Grantina-Ievina L, Andersone U, Berkolde-Pīre D, Nikolajeva V, Ievinsh G (2013) Critical tests for determination of microbiological quality and biological activity in commercial vermicompost samples of different origins. Appl Microbiol Biotechnol 97(24):10541–10554. doi:10.1007/s00253-013-4825-x
60. Higginbotham SJ, Arnold AE, Ibañez A, Spadafora C, Coley PD, Kursar TA (2013) Bioactivity of fungal endophytes as a function of endophyte taxonomy and the taxonomy and distribution of their host plants. PLoS ONE 8(9), e73192. doi:10.1371/journal.pone.0073192
61. Neubert K, Mendgen K, Brinkmann H, Wirsel SGR (2006) Only a few fungal species dominate highly diverse mycofloras associated with the common reed. Appl Environ Microbiol 72(2):1118–1128. doi:10.1128/AEM.72.2.1118-1128.2006
62. Herrera J, Khidir HH, Eudy DM, Porras-Alfaro A, Natvig DO, Sinsabaugh RL (2010) Shifting fungal endophyte communities colonize Bouteloua gracilis: effect of host tissue and geographical distribution. Mycologia 102(5):1012–1026. doi:10.3852/09-264
63. Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ (2008) Microfungi occurring on Proteaceae in the fynbos. In CBS Biodiversity Series 7. New York: Mycotaxon LTD. p. 515
64. Magyar D, Shoemaker RA, Bobvos J, Crous PW, Groenewald JZ (2010) Pyrigemmula, a novel hyphomycete genus on grapevine and tree bark. Mycol Prog 10(3):307–314. doi:10.1007/s11557-010-0703-4
65. Sommer J, Pausch J, Brundrett MC, Dixon KW, Bidartondo MI, Gebauer G (2012) Limited carbon and mineral nutrient gain from mycorrhizal fungi by adult Australian orchids. Am J Bot 99(7): 1133–1145. doi:10.3732/ajb.1100575
66. Cai L, Jeewon R, Hyde KD (2006) Phylogenetic investigations of Sordariaceae based on multiple gene sequences and morphology. Mycol Res 110(2):137–150. doi:10.1016/j.mycres.2005.09.014
67. Hartmann M, Lee S, Hallam SJ, Mohn WW (2009) Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. Environ Microbiol 11(12):3045–3062. doi:10.1111/j.1462-2920.2009.02008.x
68. Davis EC, Shaw AJ (2008) Biogeographic and phylogenetic patterns in diversity of liverwort-associated endophytes. Am J Bot 95(8):914–924. doi:10.3732/ajb.2006463
69. Hollister EB, Schadt CW, Palumbo AV, James Ansley R, Boutton TW (2010) Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. Soil Biol Biochem 42(10):1816–1824. doi:10.1016/j.soilbio.2010.06.022
70. Stielow B, Hensel G, Strobelt D, Makonde HM, Rohde M, Dijksterhuis J, Göker M (2012) Hoffmannoscypha, a novel genus of brightly coloured, cupulate Pyronemataceae closely related to Tricharina and Geopora. Mycol Prog 12(4):675–686. doi:10.1007/s11557-012-0875-1
71. Napoli C, Mello A, Borra A, Vizzini A, Sourzat P, Bonfante P (2010) Tuber melanosporum, when dominant, affects fungal dynamics in truffle grounds. New Phytol 185(1):237–247. doi:10.1111/j.1469-8137.2009.03053.x
72. Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Mol Ecol 17(16):3707–3716. doi:10.1111/j.1365-294X.2008.03848.x
73. Luangsa-Ard JJ, Ridkaew R, Tasanathai K, Thanakitpipattana D, Hywel-Jones N (2011) Ophiocordyceps halabalaensis: a new species of Ophiocordyceps pathogenic to Camponotus gigas in Hala Bala Wildlife Sanctuary, Southern Thailand. Fungal Biol 115(7): 608–614. doi:10.1016/j.funbio.2011.03.002

74. McGuire KL, Zak DR, Edwards IP, Blackwood CB, Upchurch R (2010) Slowed decomposition is biotically mediated in an ectomycorrhizal, tropical rain forest. Oecologia 164(3):785–795. doi:10.1007/s00442-010-1686-1
75. Rajulu MBG, Lai LB, Murali TS, Gopalan V, Suryanarayanan TS (2014) Several fungi from fire-prone forests of southern India can utilize furaldehydes. Mycol Prog 13(4):1049–1056. doi:10.1007/s11557-014-0992-0
76. Börstler B, Renker C, Kahmen A, Buscot F (2005) Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. Biol Fertil Soils 42(4):286–298. doi:10.1007/s00374-005-0026-9

Diversity of Fungi on Decomposing Leaf Litter in a Sugarcane Plantation and Their Response to Tillage Practice and Bagasse Mulching: Implications for Management Effects on Litter Decomposition

ORIGINALITY REPORT

18%

SIMILARITY INDEX

PRIMARY SOURCES

1	A Niswati, S Yusnaini, M Utomo, Dermiyati, M A S Arif, S Haryani, N Kaneko. "Long-term organic mulching and no-tillage practice increase population and biomass of earthworm in sugarcane plantation", IOP Conference Series: Earth and Environmental Science, 2018 Crossref	172 words —	3%
2	ynu.repo.nii.ac.jp Internet	70 words —	1%
3	Shawn P. Brown, Mac A. Callaham, Alena K. Oliver, Ari Jumpponen. "Deep Ion Torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystem", FEMS Microbiology Ecology, 2013 Crossref	68 words —	1%
4	dspace.uevora.pt Internet	64 words —	1%
5	Christopher A. Dunlap, David A. Schisler, Michael J. Bowman, Alejandro P. Rooney. "Genomic analysis of Bacillus subtilis OH 131.1 and co-culturing with Cryptococcus flavescens for control of Fusarium head blight", Plant Gene, 2015 Crossref	57 words —	1%
6	journals.plos.org Internet	46 words —	1%

7	pure.knaw.nl Internet	43 words — 1%
8	aem.asm.org Internet	36 words — 1%
9	Hui Sun, Eeva Terhonen, Andriy Kovalchuk, Hanna Tuovila et al. "Dominant Tree Species and Soil Type Affect the Fungal Community Structure in a Boreal Peatland Forest", Applied and Environmental Microbiology, 2016 Crossref	35 words — 1%
10	Meslé, M., C. Périot, G. Dromart, and P. Oger. "Biostimulation to identify microbial communities involved in methane generation in shallow, kerogen-rich shales", Journal of Applied Microbiology, 2013. Crossref	29 words — < 1%
11	Christopher J. Chizinski, Anna Peterson, JoAnn Hanowski, Charles R. Blinn, Bruce Vondracek, Gerald Niemi. "Breeding bird response to partially harvested riparian management zones", Forest Ecology and Management, 2011 Crossref	27 words — < 1%
12	Yao Huang, Nobuhiro Kaneko, Taizo Nakamori, Toshiko Miura, Yoichiro Tanaka, Masanori Nonaka, Chisato Takenaka. "Radiocesium immobilization to leaf litter by fungi during first-year decomposition in a deciduous forest in Fukushima", Journal of Environmental Radioactivity, 2016 Crossref	26 words — < 1%
13	mafiadoc.com Internet	26 words — < 1%
14	www.jstage.jst.go.jp Internet	24 words — < 1%
15	Pilloni, Giovanni, Michael S. Granitsiotis, Marion Engel, and Tillmann Lueders. "Testing the Limits of 454 Pyrotag Sequencing: Reproducibility, Quantitative	23 words — < 1%

Assessment and Comparison to T-RFLP Fingerprinting of Aquifer Microbes", PLoS ONE, 2012.
Crossref

16 eemb.ut.ee
Internet
23 words — < 1%

17 Adhikari, Keshav R., Khem R. Dahal, Zueng-Sang Chen, Yih-Chi Tan, and Jihn-Sung Lai. "Rice–wheat cropping system: tillage, mulch, and nitrogen effects on soil carbon sequestration and crop productivity", Paddy and Water Environment, 2015.
Crossref
21 words — < 1%

18 N. B. Zimmerman, P. M. Vitousek. "Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape", Proceedings of the National Academy of Sciences, 2012
Crossref
21 words — < 1%

19 www.frontiersin.org
Internet
21 words — < 1%

20 J.H.C. Woudenberg, M.F. Seidl, J.Z. Groenewald, M. de Vries, J.B. Stielow, B.P.H.J. Thomma, P.W. Crous. "Alternaria section Alternaria: Species, formae speciales or pathotypes?", Studies in Mycology, 2015
Crossref
20 words — < 1%

21 d-nb.info
Internet
20 words — < 1%

22 Bing Liu, Muhamed Jamal Hosen Talukder, Eeva Terhonen, Maija Lampela, Harry Vasander, Hui Sun, Fred Asiegbu. "The microbial diversity and structure in peatland forest in Indonesia", Soil Use and Management, 2019
Crossref
17 words — < 1%

23 bmcplantbiol.biomedcentral.com
Internet
17 words — < 1%

24 Christian Poll, Thomas Brune, Dominik Begerow, Ellen Kandeler.

	"Small-scale Diversity and Succession of Fungi in the Detritusphere of Rye Residues", Microbial Ecology, 2009 Crossref	16 words — < 1%
25	e.bangor.ac.uk Internet	16 words — < 1%
26	www.sardi.sa.gov.au Internet	16 words — < 1%
27	Aurore Stroobants, Florine Degrune, Claire Olivier, Céline Muys et al. "Diversity of Bacterial Communities in a Profile of a Winter Wheat Field: Known and Unknown Members", Microbial Ecology, 2014 Crossref	16 words — < 1%
28	Sun, Hui, Minna Santalahti, Jukka Pumpanen, Kajar Köster, Frank Berninger, Tommaso Raffaello, Ari Jumpponen, Fred O. Asiegbu, and Jussi Heinonsalo. "Fungal community shifts in structure and function across a boreal forest fire chronosequence", Applied and Environmental Microbiology, 2015. Crossref	14 words — < 1%
29	Bowman, Andrew S, Srinand Sreevatsan, Mary L Killian, Shannon L Page, Sarah W Nelson, Jacqueline M Nolting, Carol Cardona, and Richard D Slemons. "Molecular evidence for interspecies transmission of H3N2pM/H3N2v influenza A viruses at an Ohio agricultural fair, July 2012", Emerging Microbes & Infections, 2012. Crossref	14 words — < 1%
30	escholarship.org Internet	14 words — < 1%
31	doi.org Internet	14 words — < 1%
32	pub.epsilon.slu.se Internet	13 words — < 1%

33	Yang, Ching-chia, and Wataru Iwasaki. "MetaMetaDB: A Database and Analytic System for Investigating Microbial Habitability", PLoS ONE, 2014. Crossref	12 words — < 1%
34	ir.library.oregonstate.edu Internet	12 words — < 1%
35	Takashi Osono. "Decomposition of organic chemical components in relation to nitrogen dynamics in leaf litter of 14 tree species in a cool temperate forest", Ecological Research, 01/2005 Crossref	12 words — < 1%
36	Ravin Poudel, Ari Jumpponen, Megan M. Kennelly, Cary L. Rivard, Lorena Gomez-Montano, Karen A. Garrett. "Rootstocks Shape the Rhizobiome: Rhizosphere and Endosphere Bacterial Communities in the Grafted Tomato System", Applied and Environmental Microbiology, 2019 Crossref	12 words — < 1%
37	Christian Poll. "Small-scale Diversity and Succession of Fungi in the Detritusphere of Rye Residues", Microbial Ecology, 06/04/2009 Crossref	12 words — < 1%
38	B. Berg. "The influence of experimental acidification on needle litter decomposition in a Picea abies L. forest", Scandinavian Journal of Forest Research, 1986 Crossref	11 words — < 1%
39	Xu, L.. "Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing", Soil Biology and Biochemistry, 201203 Crossref	11 words — < 1%
40	www.cambridge.org Internet	11 words — < 1%
41	repository.publisso.de Internet	11 words — < 1%

42	nph.onlinelibrary.wiley.com Internet	11 words — < 1%
43	Lars Ola Nilsson. "Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization", New Phytologist, 5/2003 Crossref	11 words — < 1%
44	esa.org Internet	11 words — < 1%
45	D A Iryani, N M Risthy, D A Resagian, S D Yuwono, U Hasanudin. "Preparation and evaluation adsorption capacity of cellulose xanthate of sugarcane bagasse for removal heavy metal ion from aqueous solutions", IOP Conference Series: Earth and Environmental Science, 2017 Crossref	11 words — < 1%
46	www.ccsenet.org Internet	10 words — < 1%
47	globalsciencebooks.info Internet	10 words — < 1%
48	Manuel de Paz, Miriam E. Gobbi, Estela Raffaele. "Fallen fruits stimulate decomposition of leaf litter of dominant species in NW Patagonia shrublands", Plant and Soil, 2018 Crossref	10 words — < 1%
49	naosite.lb.nagasaki-u.ac.jp Internet	10 words — < 1%
50	Atsushi Yagioka, Masakazu Komatsuzaki, Nobuhiro Kaneko. " The effect of minimum tillage with weed cover mulching on organic daikon (var. cv. Taibyousoufutori) yield and quality and on soil carbon and nitrogen dynamics ", Biological Agriculture & Horticulture, 2014 Crossref	10 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		