



No tillage and bagasse mulching alter fungal biomass and community structure during decomposition of sugarcane leaf litter in Lampung Province, Sumatra, Indonesia

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ABSTRACT

Soil fungi are the predominant decomposers of soil organic matter (SOM). To manage SOM in tropical agricultural soils, it is important to understand the effects of agricultural management on fungal communities and their decomposition of organic matter. Our study site was located in a sugarcane plantation in Lampung Province, Sumatra, Indonesia. The objectives of this study were to determine the following: (1) the effect of conversion from conventional tillage to no-tillage farming and the application of bagasse mulch on fungal biomass, community structure, and the relative ratio of fungal to bacterial biomass (F:B); (2) the combination effect of no-tillage with bagasse mulch on these fungal parameters; and (3) possible links between these fungal parameters and the decomposition rate of sugarcane leaf litter. We measured fungal biomass and F:B by phospholipid fatty acid (PLFA) analysis, and we evaluated fungal molecular diversity and community structure by modified terminal restriction fragment length polymorphism (T-RFLP) profiling. Fungal biomass was 2-fold greater with no-tillage and 2.5-fold greater with added bagasse mulch relative to conventional (tillage without mulch) plots. On the other hand, no-tillage also increased bacterial biomass and fungal OTU (operational taxonomic unit) richness, whereas bagasse mulch increased the F:B and inhibited a specific fungal OTU. Under a combination of no-tillage and bagasse mulch, the fungal biomass was 1.7-fold greater than in conventional plots, indicating that the combination did not have an additive effect on fungal biomass. The litter mass loss rate was negatively correlated with fungal biomass, and bagasse mulch suppressed the mass loss approximately 20% less than in the conventional plots. However, the mass loss rate in no-tillage plots did not differ from that in conventional plots. Overall, our results indicated that no-tillage and bagasse mulch increased litter fungal biomass and altered the fungal communities, and these changes were reflected in the litter decomposition and soil C dynamics. Further studies are needed to clarify the relationship between litter decomposition and fungal species identity.

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1. Introduction

Soil fungi represent a major proportion of soil microbial biomass and are considered the dominant decomposers of organic matter in tropical soils (Yang and Insam, 1991; Lodge, 1993; Salamanca et al., 2006). Previous studies indicated that decomposition of organic matter is affected by the richness and community structure of

fungal species as well as biomass (Setälä and McLean, 2004; Deacon et al., 2006), and a fungal-to-bacterial biomass ratio is considered to be associated with resource stoichiometry (Strickland and Rousk, 2010). However, few studies have focused on fungal communities and their contribution to decomposition in crop lands, especially in tropical regions. Although agricultural management efforts such as no-tillage and covering fields with crop residue (mulch) have been reported to affect fungal biomass (Hendrix et al., 1986; Frey et al., 1999; Helgason et al., 2009; Carrera et al., 2007; Elfstrand et al., 2007), their impact on soil fungal diversity and community structure is not clear (Wu et al., 2007; Nishizawa et al., 2010; Gil et al., 2011). Soils contain various types of organic matter, such as plant

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litter and decomposing insects and animals; thus, the fungal community structure can vary greatly among different types of organic matter (Horwath, 2007; Thorn and Lynch, 2007; Shaheen et al., 2008; Hanson et al., 2008). Therefore, the actual impact of no-tillage and mulch on soil fungal communities and their relationship to decomposition cannot be understood simply from bulk soil. In this study, we focus on the effect of no-tillage and mulch on fungal communities in litter, which is the first step in transferring organic matter into soil.

In recent years, the implementation of no-tillage and reduced tillage systems has increased in the sugarcane plantations of Brazil and Australia to reduce soil erosion and soil organic C loss (Galdos et al., 2009; Stirling et al., 2010). Tropical agricultural lands often have problems with soil degradation resulting from the loss of organic matter (Paustian et al., 1998; Bot and Benites, 2005; Hartemink, 2006). Soil C turnover is twice as fast in tropical regions as in temperate regions, which is likely due to lower cation exchange capacities (CEC), less stabilized C, and higher temperature and precipitation, leading to faster decomposition (Six et al., 2002). In general, practicing no-till agriculture and mulching helps to increase and maintain soil organic matter (SOM) (e.g., Machado and Silva, 2001; Jimenez and Lal, 2006; de Rouw et al., 2010; Neto et al., 2010; Verma et al., 2010; Fasinmirin and Reichert, 2011; Guto et al., 2012). No-tillage prevents soil C loss due to erosion after tilling (Conant et al., 2007) and increases the proportion of macroaggregates, which results in the accumulation of SOM within the aggregate structure (Six et al., 2006). 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 (Taja and Vanderzaag, 1991; Barzegar et al., 2002), mainly by increasing C:N residue ratios and reducing soil erosion (Scopel et al., 2005; Jordan et al., 2010). Sugarcane leaf litter, which amounts to 6–8 tons dry weight/ha/year, is also an important soil input (Singh et al., 2008). Since it remains unclear the effect of different agricultural management on litter fungal communities which have a key role of SOM transition from litter to soil, our study would provide insight into predicting SOM dynamics in tropical agroecosystems.

Previous studies indicated that fungal biomass is greater under no-tillage than conventional tillage because fungal hyphal networks are not disturbed by the mechanical mixing that occurs during tillage (Beare et al., 1992; Helgason et al., 2009) and because fungal populations are enhanced by increasing the soil moisture content while increasing the soil porosity and presence of surface plant residues (Blevins et al., 1983; Hendrix et al., 1986; Frey et al., 1999). Changes in moisture conditions also alter the fungal community structure due to varying drought tolerance among fungal species (McLean and Huhta, 2000; Shi et al., 2002; Gleason et al., 2004; Robertson et al., 2006). The lack of physical soil disturbance may change the fungal community because tolerance to tillage-induced disruption of the hyphae differs among fungal species (Jansa et al., 2003; Schnoor et al., 2011). In addition, weed growth is often not suppressed under no- or minimum-tillage conditions because many weed seeds stay near the soil surface without tillage (Mohler and Callaway, 1995; Clements et al., 1996). It has been suggested that plants determine the composition and activity of a soil microbial community (Wardle et al., 1997).

Mulch also affects fungal communities, and its effect depends on the quality of the plant residue used as mulch. Low-quality resources (high C:N) favor fungi, whereas high-quality resources (low C:N) favor bacteria (Bossuyt et al., 2001), and the fungal community structure changes depending on the type of organic input (Lejon et al., 2007; Kubartova et al., 2009). For example, wheat, rye, and hairy vetch residue have been shown to promote the growth of specific fungal groups and decrease the overall fungal diversity compared to bare soil (Punja et al., 2008; Nishizawa et al.,

2010). Previous studies on bagasse amendments have focused on changes in soil chemical characteristics, crop production, and soil moisture retention (Taja and Vanderzaag, 1991; Barzegar et al., 2002; Tabarant et al., 2011).

We aimed to better understand the changes in litter fungal communities that occur as a result of no-tillage and mulch application to the soil. Our objectives during this study were to determine the following: (1) the effect of conversion from conventional tillage to no-tillage and the application of bagasse mulch on fungal biomass, fungal to bacterial biomass ratio, and fungal species richness, diversity, and community structure; (2) the combination effect of no-tillage with bagasse mulch on these fungal parameters; and (3) possible links between these fungal parameters and the decomposition rate of sugarcane leaf litter at a sugarcane plantation in Sumatra, Indonesia.

2. Materials and methods

2.1. 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 and on Alisol soil (FAO, 2001). The total precipitation amounts at this site during the dry season (May 2010 to September 2010) and the wet season (October 2010 to April 2011) were 854 and 2097 mm, respectively. The average air temperatures during the dry and wet season were 28.7 and 26.7 °C, respectively. 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 five 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 ploughed three times to depths of 20 (first), 40 (second), and 20 cm (third) in July 2010. In the mulch treatment, 80 tons (wet weight) per hectare of bagasse mulch were spread on the soil surface from August 1 to 5. Eighty tons (wet weight) per hectare of organic BBA fertilizer, consisting of five parts Bagasse, three parts Blotong (filter cake), and three parts bagasse ash, were spread prior to ploughing in the CT and CTM plots and after planting in NT and NTM plots. 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 on July 21–30, 2010. Herbicides were not applied to any of the treatments.

2.2. Soil sampling and measurement of physical and chemical conditions and weeds sampling

Field soil was collected on September 23, 2010 and January 25, 2011. Three soil samples per plot were collected using a 100 cc corer at 0–5 cm depth and thoroughly mixed. The soil pH was determined by a 1:1 soil:H₂O suspension and 1:2.5 soil:1 M KCl suspension. The soil temperature at a 5-cm depth and the volumetric water content (0–10 cm) were recorded with a HydroSense soil moisture sensor (Decagon Devices, Pullman, WA, USA). Weeds were cut at ground level from 1 m² quadrants in each plot on January 25, 2011 and were oven dried at 80 °C for 24 h to determine the dry weight. Total soil carbon (C) and nitrogen (N) and the C:N ratio of sugarcane leaf litter, bagasse and weeds were analyzed using an elemental analyzer (CN coder MT-700, Yanaco, Kyoto, Japan). For analysis of the C and N content of the weeds, the leaves of two dominant species, *Brachiaria distachya* and *Borreria latifolia*, were selected.

2.3. Decomposition rate of sugarcane leaf litter

Nylon bags (mesh size 2 mm) of 20 cm × 20 cm containing 10 g (dry weight) of brown sugarcane leaves cut to 15 cm lengths were used to measure the decomposition of sugarcane leaf litter. The bags were placed on the soil surface on September 24, 2010. In the mulch treatments, the bags were covered with bagasse. Two litterbags per treatment were collected after 124 days (January 25, 2011): one to measure mass loss and one for PLFA and T-RFLP analysis of the fungal community. The leaf litter was sorted to remove roots and soil. Litter samples were then dried at 80 °C for 24 h and weighed to estimate water content. 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 PLFA and T-RFLP analysis, the roots and soil were shaken off with a sterilized spatula, and the leaves were freeze-dried and stored at –20 °C until later analysis.

2.4. Analysis of fungal biomass and the ratio of fungal to bacterial biomass in sugarcane leaf litter

Phospholipid fatty acid (PLFA) analysis was used to assess the fungal biomass and the relative ratio of fungal and bacterial biomass. PLFAs were extracted from 0.5 g finely ground freeze-dried samples using a procedure based on those of Frostegård et al. (1991) and Niwa et al. (2008). 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 a final uptake in dichloromethane. Methyl nonadecanoate (19:0) was added to each sample as an internal standard. The fatty acid methyl esters were separated by gas chromatography with a Sherlock Microbial Identification System (MIDI, Newark, DE, USA). The fatty acids 18:2 ω 6,9 were used to estimate saprophytic fungal biomass, and 15:0iso, 15:0anteiso, 15:0, 16:0iso, 16:1 ω 7c, 17:0iso, 17:0anteiso, 17:0cyclo, 17:0, 18:1 ω 7, and 19:0cyclo ω 8c were used to estimate bacterial biomass (Frostegård and Bååth, 1996; Stahl and Klug, 1996; Frostegård et al., 2011). The ratio of fungal to bacterial PLFAs (F:B ratio) was used as an indicator of the relative ratio of fungal to bacterial biomass (Frostegård and Bååth, 1996).

2.5. Molecular analysis of fungal species richness and diversity in sugarcane leaf litter

A modified terminal restriction fragment length polymorphism (T-RFLP) method (Nishizawa et al., 2010) was used to measure fungal diversity. 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. To investigate the fungal community structure, the primer pair ITS1/LR21 was used to amplify a long DNA fragment (850–950 bp) constituting the whole ITS region and approximately 300 bp of the fungus LSU rRNA gene containing the domain 1 (D1) region (D1fLSU) (Nishizawa et al., 2010). This ITS-D1_{fLSU} rRNA region was amplified by PCR from total genomic sugarcane leaf DNA with the primers QITS1f (5'-TCCGTAGGTGAACCTGCGG-3'), labeled with quenching fluorescence, and LR21 (5'-ACTTCAAGCGTTCCCTTT-3'). The 5'-end fluorescence-labeled primer (Kurata et al., 2001) was purchased from J-Bio21 (Tsukuba, Japan). The PCR mixture (30 μ l) was prepared by combining 0.05 μ g of template DNA, 1.0 μ l of 10 pmol μ l⁻¹ primers, Takara EX Taq HS, dNTPs, and 3 μ l of optimized 10 \times EX buffer (Takara Bio Inc., Otsu, Japan) in a GeneAmp PCR System 9700 (Applied Biosystems, Houston, USA). The PCR of ITS for T-RFLP profiling was carried out under the following

conditions: 3 min at 95 °C followed by 30 cycles of 95 °C (30 s), 54 °C (45 s), and 72 °C (1.5 min).

T-RFLP was conducted as follows: the fluorescently labeled PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and eluted in a final volume of 30 μ l. Aliquots (5 μ l) of amplified ITS-D1_{fLSU} fragment were separately digested with HhaI (Takara Bio) according to the manufacturer's instructions. These labeled fragments were purified with the QIAquick Gel Extraction Kit (Qiagen). The precise lengths of the terminal restriction fragments (T-RFs) from the amplified ITS-D1_{fLSU} fragments were determined on an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). For measurement, 0.5 μ l of purified T-RF DNA was mixed with 9.25 μ l of Hi-Di formamide and 0.25 μ l of DNA fragment-length standard LIZ[®] (–250) 500 (Applied Biosystems) for standardization. This mixture was then denatured at 96 °C for 2 min and immediately chilled on ice prior to electrophoresis with an automated DNA sequencer in GeneScan mode. The lengths of the fluorescently labeled T-RFs were determined by comparison with internal standards using Peak Scanner[™] Software (version 1.0, Applied Biosystems).

The analysis produced a community profile for each sample consisting of peaks of varying height and base-pair length. Each T-RF fragment can be used as each operational taxonomic unit (OTU) to estimate the richness, evenness, and diversity of a given sample (Liu et al., 1997). T-RFs were sorted using the Ribosort package (Scallan et al., 2008) in R (v. 2.12.2), and segments shorter than 50 bp were excluded from the analysis.

2.6. Statistical analysis

The normality and heterogeneity of the data were determined with Shapiro–Wilk test and Bartlett's test, respectively. All data were log transformed when necessary to meet the assumption of normality. The main and interaction effects of tillage and mulch were assessed by a split-plot two-way analysis of variance (ANOVA) with a generalized linear model (GLM). Fungal OTU richness, Pielou's evenness, and inverse Simpson's diversity indices of fungal T-RFLP profiles were calculated using the Vegan package in R (Oksanen et al., 2011). In order to model the relationship between fungal parameters and environmental parameters and the relationship between litter mass loss rate and fungal parameter, regression models that significantly explained the variance and had the smallest Akaike information criterion (AIC) was adopted. T-RFs were analyzed using the additive main effects and multiplicative interaction (AMMI) model in the R packages agricolae and klaR (de Mendiburu, 2012) to visualize relationships and examine variance between treatments. The AMMI model combines the additive elements of ANOVA with the multiplicative elements of PCA (Gauch, 1992). AMMI analyses of T-RFLP profiles have been employed to study microbial community attributes (Culman et al., 2006, 2008; Lu et al., 2010a, 2010b; Jack et al., 2011). T-RFs that were clearly separated among the treatments by AMMI analysis were extracted, and the effects of tillage and mulch on the T-RF abundance were assessed by using split-plot two-way GLM-ANOVA. The GLM-ANOVA and regression analyses were performed using R 2.12.2 (R Development Core Team, 2011), and AMMI analysis was performed using R 2.7.2 (R Development Core Team, 2008).

3. Results

3.1. Effect of agricultural management on environmental parameters

The soil physical and chemical characteristics of the each treatment plot are shown in Table 1. At the beginning of the

Table 1
Soil physical and chemical characteristics of the treatment plots in January 2011. CT: conventional tillage, CTM: conventional tillage with bagasse mulch, NT: no tillage, NTM: no tillage with bagasse mulch.

Management	Soil pH (H ₂ O)	Soil pH (KCl)	Soil total C (%)	Soil total N (%)	Soil moisture (%)	Soil temperature (°C)	Litter moisture (%)	Weed biomass (g dry/m ²)
CT	5.64 (0.31)	4.49 (0.20)	1.08 (0.24)	0.074 (0.016)	17.4 (3.66)	29.6 (1.39)	21.6 (8.19)	142.2 (43.2)
CTM	5.53 (0.32)	4.49 (0.30)	1.31 (0.09)	0.082 (0.019)	19.0 (4.15)	28.8 (1.83)	54.4 (22.6)	19.0 (19.5)
NT	5.59 (0.20)	4.52 (0.25)	1.28 (0.20)	0.080 (0.014)	18.4 (5.00)	29.3 (1.73)	52.8 (7.28)	290.8 (183.1)
NTM	5.51 (0.14)	4.42 (0.22)	1.18 (0.13)	0.075 (0.017)	21.4 (4.18)	28.8 (1.77)	59.7 (21.6)	248.5 (91.07)
GLM-ANOVA F-test (P-values)								
Tillage	<0.01	0.39	0.20	0.80	0.68	0.78	<0.05	<0.01
Mulch	0.24	0.64	0.39	0.85	0.07	0.36	<0.01	0.19
Tillage × mulch	0.85	0.67	<0.05	0.37	0.54	0.91	0.06	<0.01

Values in the upper part of the table represent means (s.d.m.; $n = 5$).

Values in bold are significant ($P < 0.05$).

experiment, the total C content (% dry mass) was 47.5 for sugarcane leaf litter and 47.7 for bagasse, and the C:N ratio was 73 for sugarcane leaf litter and 142 for bagasse (data not shown). We found no differences in soil pH (KCl), soil total N, soil moisture, or soil temperature among treatments. Soil pH (H₂O) decreased under no-tillage conditions ($P < 0.05$). On the other hand, soil total C was affected positively by bagasse mulch but only under conventional tillage, and by no-tillage but only without mulch (tillage × mulch interaction, $P < 0.05$). Litter moisture increased under no-tillage ($P < 0.05$) and bagasse mulch ($P < 0.01$). Weed biomass increased under no-tillage ($P < 0.05$), and the positive effect of no-tillage on weed biomass was stronger under bagasse mulch (tillage × mulch interaction, $P < 0.01$) (Table 1). The total C and C:N ratio of weeds were 39.4 (average of two species, s.d. = 2.3) and 19 (s.d. = 2.8), respectively (data not shown).

3.2. Fungal biomass and the ratio of fungal to bacterial biomass in sugarcane leaf litter

The amount of fungal PLFA was 2.5-fold greater in CTM plots and 2-fold greater in NT plots than in CT plots (Table 2). ANOVA indicated a positive effect of bagasse mulch on the fungal PLFA ($P < 0.05$) (Table 2). There was, however, a tillage × mulch interaction effect ($P < 0.01$): the positive effect of bagasse mulch on fungal biomass was pronounced only under conventional tillage (Table 2).

The fungal to bacterial PLFAs ratio (F:B ratio) was higher in the CTM plots than in the three other treatment plots (Table 2). The positive effect of bagasse mulch on F:B ratio was detected only under conventional tillage (tillage × mulch interaction, $P < 0.05$). F:B ratio was also negatively correlated with weed biomass (Fig. 1).

3.3. Fungal richness, evenness, diversity, and community structure

The T-RFLP profiles of fungi were compared among treatments (Table 2 and Fig. 2). In total, 285 fungal OTUs were detected across

all of the samples. The ANOVA output demonstrated that the number of fungal OTUs was increased by no-tillage ($P < 0.05$), and we observed a marginal increase in Simpson's diversity under no-tillage, whereas the evenness did not differ significantly among the treatments (Table 2).

The predominance of specific OTUs (relative abundance of >9% of the total profile) in each of the treatment plots were as follows: two OTUs of 110 and 445 bp in the CT plot, one OTU of 110 bp in CTM, three OTUs of 110, 444 and 445 bp in NT, and one OTU of 110 bp in the NTM plot (Fig. 2). A 110-bp OTU was affected negatively by tillage, but only under bagasse mulch treatment (tillage × mulch interaction, $P < 0.05$). A 445-bp OTU was affected negatively by bagasse mulch ($P < 0.05$). A 444-bp OTU was detected in the NT plots but not in any other treatment plots. In addition, a 317-bp OTU was detected in the NT and NTM plots but was not detected in the CT plots, and its abundance was lower in the CTM plots than in the NT and NTM plots. The abundance of the 317-bp OTU was affected positively by no-tillage ($P < 0.01$). According to an AMMI analysis of T-RFLP profile, the fungal community structure clearly differed among the treatments ($P < 0.05$). The first principal component (PC1) explained 57.2% of the total variation, and the first and second principal components (PC2) explained 91.3% of the variation. The 444-bp OTU interacted positively with NT plots. The 445-bp OTU interacted positively with CT and NT plots. The 110 and 317-bp OTUs interacted positively with NT and NTM plots (Fig. 3).

3.4. Relationships between the decomposition rate of sugarcane leaf litter and fungal community

The litter mass loss rate was lowest in the CTM plot, followed by the NTM, NT, and CT plots (Table 2). ANOVA showed that litter mass loss rate was affected negatively by bagasse mulch ($P < 0.05$). We also found a significant negative correlation between the mass loss rate and the amount of fungal PLFA ($P < 0.001$) (Fig. 4), while F:B ratio, OTU richness, and diversity were not significant correlated with the mass loss rate (data not shown).

Table 2
Effect of agricultural management on fungal PLFA, F:B ratio, fungal OTU richness, fungal diversity, and litter mass loss.

Management	Fungal PLFAs (µg/g litter)	F:B ratio	Fungal OTU richness	Fungal evenness	Inverse Simpson's index	Proportion of ash free mass loss (%)
CT	33.6 (12.5)	0.29 (0.09)	32.8 (12.2)	0.69 (0.10)	6.27 (1.81)	64.0 (9.7)
CTM	91.2 (32.7)	0.58 (0.19)	35.4 (12.8)	0.66 (0.05)	5.77 (2.16)	44.9 (11.3)
NT	68.1 (18.9)	0.27 (0.07)	43.6 (8.4)	0.70 (0.08)	8.04 (2.81)	63.9 (9.2)
NTM	56.5 (22.8)	0.31 (0.10)	38.8 (6.5)	0.69 (0.07)	7.16 (2.29)	57.0 (12.1)
GLM-ANOVA F-test (P-values)						
Tillage	0.98	<0.05	<0.05	0.55	0.18	0.24
Mulch	<0.05	<0.01	0.77	0.62	0.53	<0.05
Tillage × mulch	<0.01	<0.05	0.34	0.73	0.87	0.16

Values in the upper part of the table represent means (s.d.m.; $n = 5$).

Values in bold are significant ($P < 0.05$).

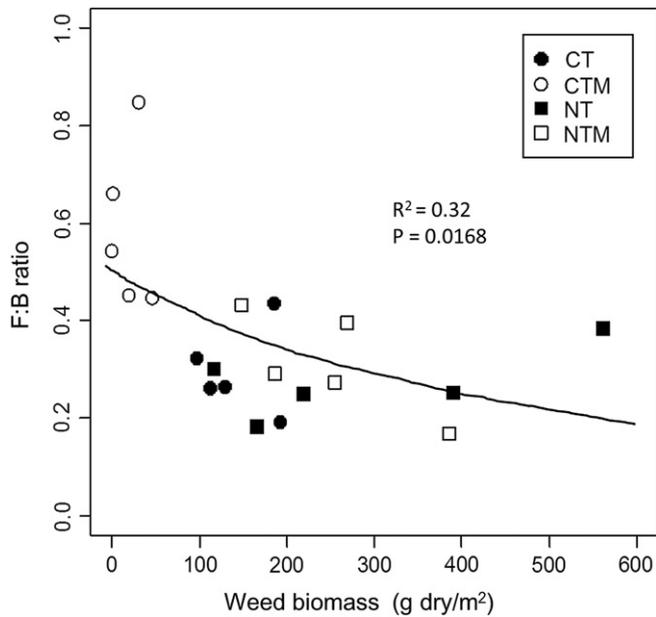


Fig. 1. Relationships between weed biomass and F:B ratio. Symbols represent single observations.

4. Discussion

4.1. Effect of converting conventional tillage to no-tillage on litter fungal communities

Our fungal PLFA and T-RFLP analysis revealed that converting from conventional to no-tillage increased fungal biomass, fungal OTU richness, and the number of predominant fungal OTUs. Soil fungal communities are known to be more sensitive to soil physical disturbance than bacteria (Hossain and Sugiyama, 2011). van der Wal et al. (2006) reported that the major reason for the short-term increase of soil fungal biomass after land abandonment was due to cessation of human interventions. Wu et al. (2007) differentiated soil fungal communities based on their resistance to disturbance events, and Plassart et al. (2008) found that there was a strong positive relationship between soil fungal genetic diversity and aging grassland converted from tillage agricultural land. We focused on the fungal community in the litter layer, where no direct tillage effects can be expected. Beare et al. (1992) and Beare et al. (1993) showed that fungal biomass in surface litter on no-tillage soil was greater than that on conventional tillage and that litter fungal biomass was decreased in fungicide-treated soil relative to that in control soil. We also confirmed that no-tillage practice increased litter fungal biomass and diversity. This finding indicates that fungal communities in soil as well as in litter are influenced by soil physical and biological disturbance. Therefore, a lack of soil physical disturbance seemed to be favorable for fungal biomass and diversity in the litter layer.

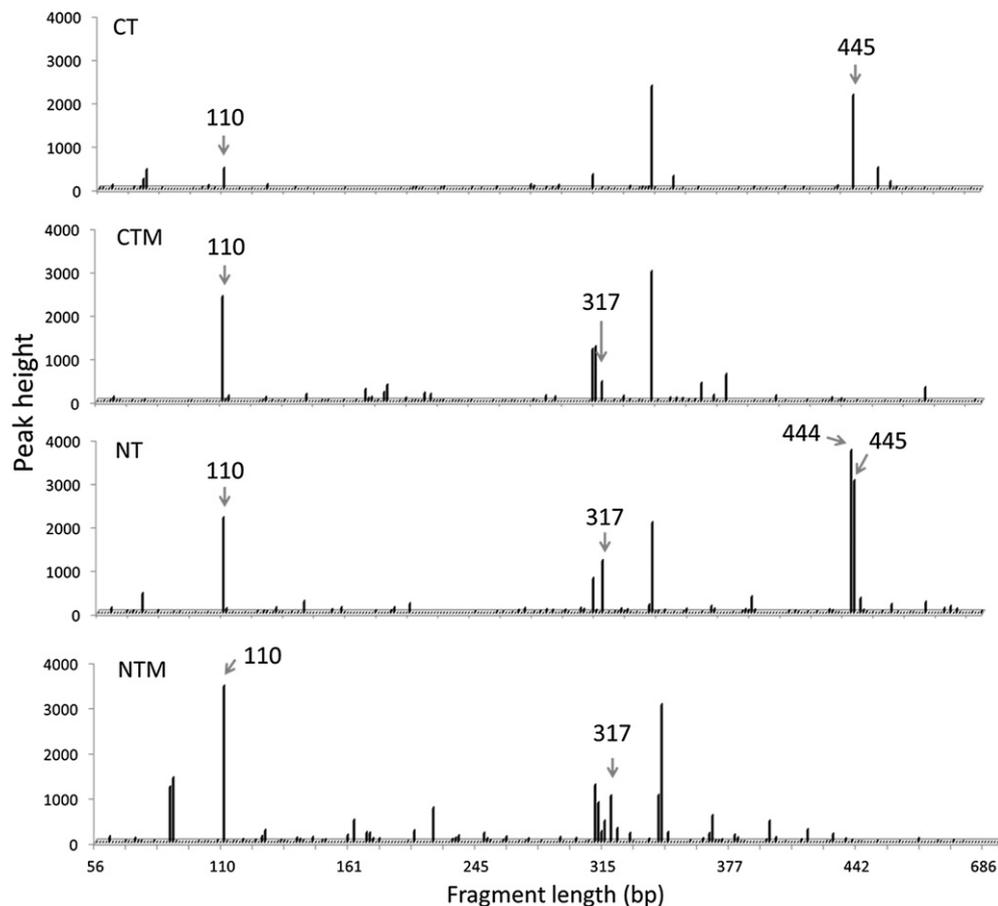


Fig. 2. T-RFLP profiles of fungal communities in sugarcane leaf litter from different agricultural management treatments. Graphs were generated from the mean peak height of T-RFs of PCR amplicons digested with HhaI restriction endonuclease for the fungi ITS region. The size (base pairs) of T-RFs that differ significantly between treatments is indicated.

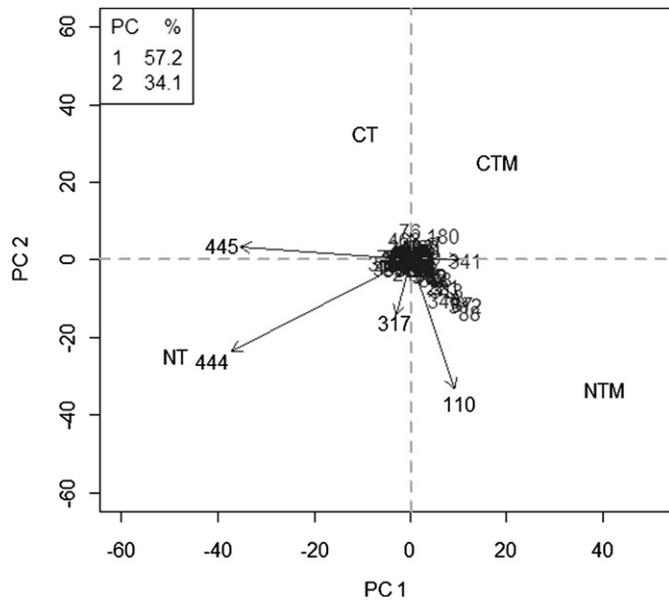


Fig. 3. AMMI biplot of T-RFLP profiles using T-RF relative abundance data obtained from the different treatments in the sugarcane leaf litter. The percentages indicate the proportions of variation explained by the first and second ordination axes. Arrows indicate the distribution of T-RFs.

Although there was no clear relationship between weed biomass and microbial parameters except for the F:B ratio, it is worth noting that a higher weed biomass was confirmed in no-tillage treatment. We found that tillage suppressed the weed growth, which is consistent with the findings of Mohler and Callaway (1995) and Clements et al. (1996). In our experimental site, weeds were slashed on the soil surface manually at 2, 3, and 6 months after planting, and vines were retrieved by hand at 7, 9, and 12 months after planting. Wardle et al. (1999) reported that weed residue increased the soil microbial biomass and soil respiration because weeds contain a higher proportion of available nutrients than crop residues. Therefore, the high weed biomass in the no-

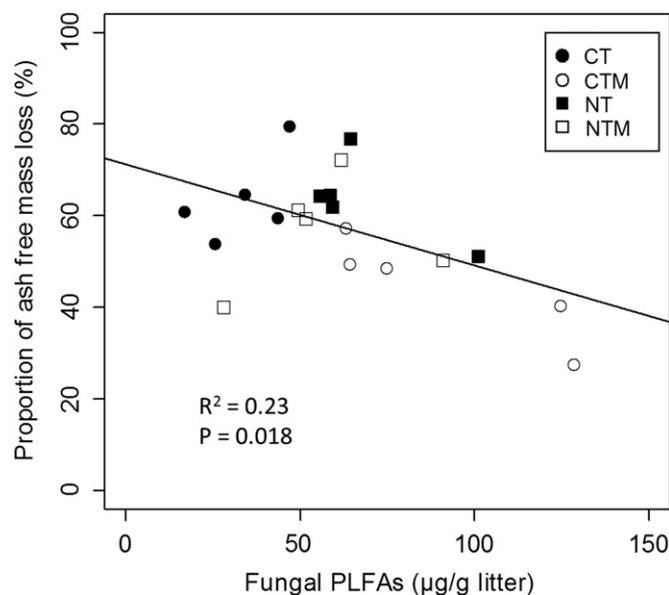


Fig. 4. Relationships between litter mass loss and fungal biomass. Symbols represent single observations.

tillage treatment might have added greater C and nutrients for fungi and also helped to increase litter moisture in the no-tillage plots. On the other hand, the F:B data implies that weeds encouraged bacterial growth, unlike bagasse which stimulated fungal growth but not bacterial growth. In addition, the fungal PLFA and F:B data in NT and NTM suggest that mulch additions to NT soil may inhibit bacterial populations. In general, it is considered that bacteria require more N per unit biomass C accumulation than fungi (De Deyn et al., 2008). Therefore, these results indicated that weeds increased bacterial biomass favorably because weeds contain greater N than bagasse.

Furthermore, the no-tillage plots we sampled contained 2–5 weed species (data not shown). The diversity of weed plants is believed to increase fungal diversity by one of two hypothesized routes: the “productivity–diversity hypothesis,” which proposes that the availability of growth-limiting resources limits the diversity of biotic communities and “the plant diversity hypothesis,” which proposes that greater plant species richness increases the range of organic substrates entering the soil, thus creating a niche space to be filled by a greater array of heterotrophic microorganisms (Waldrop et al., 2006). The higher plant diversity may create a greater chemical heterogeneity, which over time maintains greater microbial diversity through resource partitioning (McGuire et al., 2010).

4.2. Effect of bagasse mulch on litter fungal communities

The remarkable increase in litter fungal PLFA and F:B ratio under bagasse mulch may have resulted from the application of organic matter with a high C:N ratio. It has been reported that fungi have a lower N demand and use C more efficiently than bacteria (Keiblinger et al., 2010; de Vries et al., 2011); thus, fungal-dominated food webs occur in sites with a high organic matter content and low resource quality (Coleman et al., 1983; Allison et al., 2005), and the fungal-to-bacterial biomass ratio generally increase with increasing C:N ratios in soil (Fierer et al., 2009).

Bagasse mulch did not affect the OTU richness, but it inhibited a specific fungal OTU that was dominant in no-mulch treatments (Figs. 2 and 3). A differential response of fungal communities to substrates may be masked by microbial detection methods that lump the communities together (Mäder et al., 2002). Our results also illustrate the importance of quantifying species composition within a community in addition to measuring overall abundance and species diversity. The 445-bp OTU was the dominant fragment in the no-mulch treatment, and it was not present in the mulch treatment. There are two possible reasons why the 445-bp OTU was not detected in bagasse mulch treatment: 1) the chemical composition of bagasse inhibited specific fungi or 2) species that prefer bagasse suppressed other species by interspecific competition. There was no singly dominant OTU in the bagasse mulch treatment compared to the other treatments (Figs. 2 and 3). Therefore, it is more likely that bagasse inhibited the growth of fungi.

4.3. Combination effect of no-tillage and bagasse mulch on fungal communities

Under no-tillage, however, bagasse application did not affect fungal biomass (Table 2). Because there was an increase in fungal biomass during no-tillage, the application of bagasse could not further increase the fungal biomass. This outcome might be related to the carrying capacity (the maximum population that the environment can support) (Nannipieri et al., 1983). When fungal and bacterial PLFA was converted to microbial biomass C by multiplying it with the mean fungal biomass C/18:2ω6,9 ratio of 107 and the

mean bacterial C/bacterial PLFAs ratio of 4.7 (Jørgensen and Wichern, 2008), the mean microbial biomass values C as percentages of litter C would be 3.1, 7.9, 6.5, and 5.2% in the CT, CTM, NT and NTM plots, respectively. Biomass C generally comprises approximately 1–5% of soil organic C (Jenkinson and Ladd, 1981). Therefore, it is possible that the fungal biomass except in CT plot had already reached the carrying capacity.

The other notable result is that the positive effect of bagasse mulch application on the F:B ratio was not strong under no-tillage treatment relative to tillage treatment. We also found that the application of bagasse mulch to the conventional tillage soil effectively inhibited the weed growth, as in many previous studies showing that residue mulch limits weed development (Bond and Grundy, 2001), whereas it was not effective under no-tillage. As mentioned above, weeds might have increased bacterial population favorably, possibly because weed residue supplied N to bacteria. Thus, there was not much difference in the F:B ratio between no-tillage with and without bagasse mulch treatments.

4.4. Relationship between fungi and the litter decomposition rate

The litter decomposition rate did not differ between conventional tillage and no-tillage treatment, even though the litter moisture content and fungal and bacterial biomass were significantly greater under no-tillage. A possible reason for this finding is that repeated drying and wetting enhances C and N mineralization (Miller et al., 2005; Borken and Matzner, 2009). Unlike litterbags in the no-tillage treatment that were covered by weeds, litters in exposed litterbags in the CT treatment may have been more strongly affected by precipitation.

Wardle et al. (1993) measured the decomposition rate of rye residue under sawdust mulch (C:N = 400:1) and reported that the mulch increased the decomposition rate by increasing the moisture content of the underlying rye residue. Conversely, we found that the decomposition rate of sugarcane leaf litter was suppressed by bagasse mulch, despite the higher litter moisture content. As mentioned above, fungi dominate in environments with lower available nutrients and more carbon. However, under N limitation, the addition of inorganic N increases fungal metabolic activity and promotes litter decomposition (Boberg et al., 2008). This finding suggests that fungal metabolic activity is suppressed by resources with a high C:N ratio, as Schneider et al. (2012) revealed that litter nutrient content and the stoichiometry of C:N:P affect the fungal productivity of extracellular enzymes. Meanwhile, under no-tillage treatment, the effect of the bagasse application was less strong than in bagasse mulch with conventional tillage treatment. This effect may be due to N-rich weeds promoting fungal metabolic activity.

Furthermore, we suspected that changes in the fungal community structure influence the rate of decomposition. The fungal contribution to the decomposition rate can be often explained by the fungal community structure rather than by the fungal biomass or the fungal to bacterial ratio. For example, Deacon et al. (2006) found that infrequently isolated fungi during the litter incubation were potentially more active in decomposition than the frequently isolated taxa. Setälä and McLean (2004) indicated that CO₂ production increased as fungal diversity increased in the species-poor end of the diversity gradient. There is also evidence for facilitation and competition among microbial taxa that co-occur on decomposing litter, and the traits of fungal species are one of the key drivers of litter decomposition rates (Allison, 2012). Fontaine et al. (2003) suggested that fungi can be divided into functional groups that decompose different types of organic matter: fast-growing *r*-strategists specialized in the utilization of easily available resources and slow-growing *k*-strategists are able to decompose more recalcitrant compounds. España et al. (2011) reported

that the addition of maize residues promoted slow-growing fungal decomposers, and the addition of soybean residues promoted fast-growing fungal decomposers. Fast-growing opportunistic fungi are stimulated by easy accessible C sources and high N availability (Chigineva et al., 2009; Poll et al., 2010). In addition, Schneider et al. (2012) demonstrated that active part of fungal community is changed by litter quality. These trends indicate that bagasse with a higher C:N substrate should inhibit fast-growing fungal decomposers, which we hypothesize is a characteristic of the 445-bp OTU that was found in the no-mulch treatment but not in the mulch treatment. This finding suggested that community structures are equally important in describing their functional attributes. Future efforts to discriminate the fungal species and their physiology are needed to provide further information on the relationships between fungal community structure and decomposition.

5. Conclusion

Data from this study demonstrate that no-tillage increased fungal biomass, fungal richness, and the number of dominant fungi, but it did not change the F:B ratio or the litter mass loss rate. Bagasse mulch increased fungal biomass in the conventional tillage but not the no-tillage system. Overall, the positive gain in fungal biomass was greater when the conventional tillage was amended with mulch than when transitioned to no-tillage. In addition, bagasse increased the F:B ratio, inhibited a specific fungal OTU and suppressed the decomposition rate of sugarcane leaf litter. These changes were associated with high C:N bagasse. Meanwhile, the combination of no-tillage and bagasse mulch did not have an additive effect on fungal biomass. These findings contribute significantly toward an understanding of the specific changes in soil fungal communities and fungal biomass in response to agricultural management. Further studies are needed to clarify the relationship between the litter decomposition and fungal species identity.

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