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ORIGINAL PAPER

Shifts in the composition and potential functions of soil microbial communities responding to a no-tillage practice and bagasse mulching on a sugarcane plantation

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Abstract This study examined the effect of conversion from a conventional tillage (CT) to a no-tillage (NT) practice and of bagasse mulching (M) on soil microbial community composition and potential functions, using phospholipid fatty acid (PLFA) analysis and shotgun metagenome sequencing. Our results showed that both the NT and the M treatment increased microbial PLFAs. The shotgun sequencing results suggested that the functional profiles are more resistant to agricultural managements than to community compositions, which supports the hypothesis of the functional redundancy of soil microbial communities. However, some metabolism-related sequences were significantly affected by different treatments. The percentage of sequences related to metabolism of carbohydrates, especially saccharide groups, was significantly higher in the CT soils than in NT and M soils, which may be linked to lower carbon (C) availability in CT soils. Compared with CT, the NT had higher alpha diversity and more sequences related to DNA metabolism, which may be associated with higher nutrient availability. On the other hand, the M treatment decreased the percentages of sequences related to the metabolism of amino acids and derivatives, which may be due to the limited nitrogen (N) because of the high C/N ratio of bagasse. We also observed interaction effects of the NT and M treatments; although both the NT and M treatments increased the relative abundance of Proteobacteria, this variable in NT+M soils was not higher than in each single treatment. Overall, our findings suggest that the microbial communities change their composition and functionality in response to the NT and M treatments, and these shifts have the potential to affect important soil processes that sustain crop productivity, such as C sequestration and major nutrient cycles.

Keywords Metagenomics · Tropical agroecosystem · Residue management · Conservation tillage · Functional diversity

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Introduction

In tropical areas, organic matter-rich surface soil has been rapidly lost in the conversion from forest to agricultural land (Labrière et al. 2015). Soil organic matter (SOM) losses are typically correlated with significant nutrient depletion (Giardina et al. 2000; Kauffman et al. 1995; Kimetu et al. 2008) and, as a consequence, crop yields decline dramatically (Lal 2006). Therefore, the restoration of SOM in agricultural land is crucial for crop productivity, especially in the highly weathered soils of the humid tropics (Fernandes et al. 1997; Tiessen et al. 1994).

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In Sumatra, Indonesia today, nearly half of the forest area has been lost since 1990, mainly due to the development of monoculture plantations and agro-industrial estates (Margono et al. 2012). In recent years, the no-tillage (NT) practice has been increasingly adopted by Indonesian farmers in areas where there is severe soil degradation (Utomo 2014). The NT farming is considered to be a promising farming system to prevent soil degradation in various regions (Holland 2004; Scopel et al. 2012; Wang et al. 2007). The NT practice can lead to accrual of much of the soil carbon (C) lost during tillage (Conant et al. 2007). In addition, it was reported that active C content was increased under short-term conservation tillage (Melero et al. 2009), which indicates that soil C quality is also affected by the NT practice. However, it remains unclear what effect the NT practice has on composition of soil microbial communities and their functions that mediate soil processes, such as nutrient cycling and C sequestration, in tropical agricultural systems.

Crop residue mulching is another important practice for increasing SOM content and preventing soil erosion. In sugarcane plantations, bagasse (sugarcane fibers from which the juice has been extracted) mulching has increased SOM content (Taja and Vanderzaag 1991). Mulching affects soil microbial community composition, and its effect strongly depends on the quality of the substrate. For example, low-quality resources (high C/N) favor fungi, whereas high-quality resources (low C/N) favor bacteria (Bossuyt et al. 2001). It has already been demonstrated that bagasse mulching increased the fungal biomass and fungal-to-bacterial ratio (F/B) of sugarcane leaf litter at the intermediate stage of decomposition under conventional tillage (CT) conditions (Miura et al. 2013). However, under the NT conditions, applying bagasse did not increase the fungal biomass or the F/B ratio. This result indicates that the effect of bagasse on composition of microbial communities differs between tillage and the NT systems. Therefore, to develop effective conservation management practices for sugarcane plantations, further soil microbial community surveys are needed.

The metagenomic approach has a potential to capture microbial taxonomic diversity and a large variety of enzyme-encoding genes in the soil (Myrold and Nannipieri 2014). Thus, this approach helps to determine how soil microbial communities shift their compositions and functionality in response to agricultural management practices. However, this metagenomics approach has rarely been applied in tropical agricultural soils, especially in Southeast Asia. Our study aimed to investigate the effect of the NT treatment, the M treatment, and their combination on composition of soil microbial communities and potential functions in a sugarcane plantation in Lampung Province, Sumatra, Indonesia. Several metagenomics studies showed that agricultural intensification such as tillage and fertilization had less effect on soil microbial functional profile than the microbial composition, which indicates that soil microbial functionality might have a buffering capacity aiming at minimizing the impact of agricultural practices (Pan et al. 2014; Souza et al. 2015; Wood et al. 2015). On the other hand, it has been shown that the abundance of genes related to C and nitrogen (N) cycling was significantly correlated with soil variables, which indicates that environmental variables could be important in shaping microbial functionality (Delmont et al. 2014; Fierer et al. 2012; He et al. 2010). We therefore hypothesized that the NT and bagasse mulching alter microbial functionality in association with changed soil properties.

Materials and methods

Site description

The experimental site was established in 2010 within a large area (approximately 25,000 ha) of a commercial sugarcane plantation in Sumatra, Indonesia (4° 40′ S, 105° 13′ E, altitude c.a. 45 m). The main soil type of the experimental area is Alisol (FAO 2001). We used a split-plot design with soil tillage as the main factor and bagasse mulching as a secondary factor. The treatments were CT, CT with bagasse mulching (CTM), NT, and NT with bagasse mulching (NTM) repeated across five replicate blocks. In this study, the fifth replicated plot of each treatment was omitted to reduce the cost of DNA sequencing. Each plot was 25×25 m with a 5-m buffer zone adjacent to the road. The CT treatment plots were plowed three times to depths of 20 (first), 40 (second), and 20 cm (third) on July. Eighty tons (wet weight) per hectare of organic fertilizer (bagasse/filter cake/ash=5:3:1) were spread prior to plowing in the CT and CTM treatments and after planting in the NT and the NTM treatments. This organic fertilizer contains 65 % of water and 10.1 ton C, 240 kg N, 4 kg P, and 14 kg K ha^{-1}. For the mulch treatments, 80 tons (wet weight) per hectare of bagasse (56 % of water content; 16.8 ton C ha^{-1}; 118–5–18 kg NPK ha^{-1}) was spread on the soil surface on August. Inorganic fertilizers (120–80–180 kg NPK ha^{-1}) were applied in all treatments at the time of planting. Herbicides were not applied to any of the treatments.

Soil sampling and analysis of soil and crop yield

Field soil was collected from each plot in July 2011 and July 2013 prior to sugarcane harvesting. Although we collected soil samples at 0–5, 5–15, and 15–25 cm depth, we only used 0–5 cm soil samples in this study because total soil C and N content had significant differences among treatments at 0–5 cm, but not at 5–15 and 15–25 cm depth in July 2013 (Arai et al. unpublished data). Three soil samples per plot were collected using a 100-cc corer to a depth of 5 cm, after the removal of any surface litter, and samples were mixed thoroughly. Samples were immediately returned to the laboratory.

Roots were removed by hand, and soil moisture content was measured gravimetrically using 1 g of samples of moist soil, which was oven-dried at 105 °C for 24 h. Soil pH was measured at a 1:5 (*v*/*v*) ratio of soil and deionized water. Total soil C and N were determined using an elemental analyzer (CN corder MT-700, Yanaco, Kyoto, Japan). Exchangeable potassium (K), calcium (Ca), and magnesium (Mg) were extracted with ammonium acetate (1 M) at pH 7 with a freeze-dried soil to solution ratio of 1:10 and then determined by ICP-AES (ICPE-9000; Shimadzu, Kyoto, Japan). Soluble aluminum (Al) was extracted with 1 M KCl with a 1:10 ratio of freeze-dried soil to solution and then determined by ICP-MS (Agilent 7500c; Agilent Technologies, Tokyo, Japan). Available phosphorus (P) was determined using the Bray II method and was measured with a spectrophotometer (PD-303; APEL, Saitama, Japan). Sugarcane samples were collected from 6.25 m^2 in the middle of each plot, and the dry weight and sucrose content of the sugarcane stems were measured.

PLFA analysis of soil microbial communities

A phospholipid fatty acid (PLFA) analysis was used to assess the biomass of fungi, bacteria, and actinobacteria. Immediately after soil sampling and root removal, a soil subsample was freeze-dried for PLFA analysis. PLFAs were extracted from 8 g of finely ground freeze-dried samples using a procedure based on that of Frostegård et al. (1991). 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 transesterification with a 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 on a gas chromatograph with the Sherlock Microbial Identification System (MIDI, Newark, DE, USA). As previously reported (Frostegård and Bååth 1996; Stahl and Klug 1996; Zelles 1999), iso-PLFAs and anteiso-branched PLFAs were used as indicators of Gram-positive bacteria, and monounsaturated and cyclopropyl PLFAs were used as indicators of Gram-negative bacteria. We used 10-methyl fatty acids as the marker for actinobacteria. The fatty acids 18:2ω6 were used to estimate saprophytic fungal biomass (Frostegård et al. 2011).

Shotgun metagenome sequencing and data processing

To obtain taxonomic and functional information about the sampled soil microbial communities, high-throughput sequencing was conducted using an Ion Torrent Personal Genome Machine (PGM) (Life Technologies, Tokyo, Japan). DNA was isolated from 3 g of frozen soil samples using the MO BIO PowerSoil DNA Isolation kit (Mo Bio Laboratories, CA, USA) 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, Kanagawa, Japan). The DNA samples were purified using a NucleoSpin gDNA Clean-up kit (Macherey-Nagel, Düren, Germany) prior to storage at −20 °C. Purified genomic DNA was sheared for 15 min using the Ion Xpress™ Plus Fragment Library Kit, following the manufacturer's instructions (Life Technologies). Further library preparation was performed using the Ion Plus Fragment Library Kit (Life Technologies), following manufacturer's instructions. Size selection of the library was performed using an E-gel 2 % agarose gel (Life Technologies), resulting in a median fragment size of approximately 200 bp. The concentrations of the prepared libraries were determined by quantitative PCR using the Ion Library Quantitation Kit (Life Technologies). 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). Emulsion PCR to incorporate the library to the sequencing beads was performed using the Ion OneTouch instrument with an Ion OneTouch Template 200 Kit (Life Technologies). Finally, the library sample was sequenced on an Ion Torrent Personal Genome Machine using an Ion 318 chip and an Ion PGM 200 sequencing Kit (Life Technologies), following the manufacturer's instructions. The resulting sequencing data sets were uploaded to Metagenome Rapid Annotation using the Subsystem Technology (MG-RAST) server (http://metagenomics.nmpdr.org/) and were checked for low-quality reads. The sequence data after omitting the low-quality reads were sub-sampled at random to 504,416 sequences per sample (the smallest sample size) by using the Mothur pipeline (v. 1.32.1; Schloss et al. 2009) to reduce bias associated with different numbers of reads in the different samples (Gihring et al. 2012). The sub-sampled sequence data were then uploaded to MG-RAST for dereplication, annotation, assignment of metabolic function, and phylogenetic identification, as described previously (Meyer et al. 2008). Determination of rarefaction and alpha diversity of annotated species was performed within MG-RAST by applying the "Best Hit Classification" option using the M5NR database as a reference with the following settings: maximum E-value cut-off of 1×10^{-5}, minimum identity of 60 %, and minimum alignment length of 15 amino acids for proteins and 15 base pairs for RNA. The alpha diversity estimate is a single number (the antilog of the Shannon diversity index) that summarizes the distribution of species-level annotations in a dataset. The phylogenetic origin of the metagenomics sequences was projected against the M5NR database with the same cut-off mentioned above. The functional profiles were annotated according to SEED subsystems with the same cut-off threshold mentioned above. The

sequences are publically accessible through the MG-RAST server with the ID numbers shown in Table S1.

Statistical analysis

All statistical analyses were performed using R software version 3.1 (R Development Core Team 2009). A split-plot two-way analysis of variance (ANOVA) with a generalized linear model (GLM) was used to examine the effects of tillage, mulching, and their possible interactions on soil chemical, microbial PLFAs, taxonomic and functional profiles. When a significant interaction effect was observed in the ANOVA, Tukey's post hoc comparisons were performed. Nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis metrics was applied to visualize the samples based on the taxonomic and functional profiles. NMDS ordination plots were created using the *metaMDS* function in the R vegan package, which incorporated a Wisconsin double standardization of relative abundances of annotations within each taxonomic or functional category. The similarity of treatments was calculated based on the Bray-Curtis distance metrics between the taxonomic and functional profiles with permutational multivariate analyses of variance (PERMANOVA) (Anderson 2001). These analyses were conducted using the *Adonis* function of the R vegan package (Oksanen et al. 2013) with 999 permutations. Spearman's correlations were calculated between the microbial and soil environmental parameters to identify highly correlated variables in each year.

Results

Soil properties

There was no significant difference in soil water content between treatments in the first year of the experiment but was significantly increased by the M treatment in the third year (Table 1). There was no significant difference in soil pH between treatments in either experimental year. Soil C and N content were significantly increased by the NT treatment in both years. However, there was a tillage × M interaction on soil C content in the third year; only the CT treatment statistically differed from the other treatments, according to the post hoc comparisons. The soil C/N ratio was increased by the NT and M treatments in the first year. Additionally, there was a tillage × M interaction on the C/N ratio; only the CT treatment was statistically different from the other treatments, according to the post hoc comparisons. In the third year, however, the soil C/N ratio was decreased by the NT treatment but was increased by the M treatment. Exchangeable cations (Ca and Mg), available P, and soluble Al content were not significantly different between treatments in the first year. However, there were significant differences between treatments in the third year; exchangeable Ca content was significantly increased by the NT treatment, but there was an interaction effect of tillage × M treatment. According to the post hoc multiple comparisons, the NT treatment had a significantly higher exchangeable Ca content than CT and CTM, and the CTM treatment had a significantly higher exchangeable Ca content than the CT treatment. In addition, there was no significant difference between the NTM and CTM treatments. Exchangeable Mg content was significantly increased by the NT and M treatments in the third year. Exchangeable K content was not affected by treatment type. Available P content was increased by the NT treatment, and the soluble Al content was decreased by NT treatment in the third year.

The sugarcane yield and sugar amount were decreased by the NT treatment in the first year of the experiment, but there were no statistical differences between treatments in the third year (Table S2). The sucrose content of sugarcane was not statistically different between treatments.

Soil microbial PLFAs

The concentrations of fungal PLFAs were significantly increased by the M treatment in the first year of the experiment (Fig. 1). In the third year, however, there was no statistical difference in fungal PLFAs between treatments. The concentrations of bacterial PLFAs including actinobacterial PLFAs were significantly affected by tillage, but there was an interaction effect of tillage × M treatments in the first year. The post hoc multiple comparison showed that the CT treatment had significantly lower bacterial PLFA content than other treatments. In the third year, there was a significant positive effect of the M treatment on bacterial PLFA content. The F/B ratio was significantly affected by the M treatment, but there was an interaction effect of tillage × M treatment in the first year; the CTM treatment had a significantly higher F/B ratio than the CT and NTM treatments but not statistically different from the NT treatment, according to the post hoc multiple comparison. Conversely, in the third year, there was no significant difference in the F/B ratio between treatments. The Gram-positive/Gram-negative bacterial PLFA ratio was significantly decreased by the M treatment in the first year, whereas there was no significant difference of the Gram-positive/Gram-negative bacterial PLFA ratio between treatments in the third year.

Shotgun metagenome characteristics

The 32 metagenomes comprised a total of 2.7 billion bp contained in 16.1 million reads (Table S1). Mean lengths of the sequences ranged from 107 to 180 bp. MG-RAST annotation to the M5NR database identified 80–91 % of the predicted protein-coding regions for the 32 sample metagenomes. The functional diversity, calculated by the number of functional categories divided by the metagenome size, ranged from 18

Table 1 Soil chemical properties

Treatment	Moisture (%)		pH (H_2O)		Total C (g kg^{-1} dry soil)		Total N (g kg^{-1} dry soil)		C/N		Exchangeable						Available P (mg kg^{-1} dry soil)		Soluble Al (mg kg^{-1} dry soil)	
											Ca (mg kg^{-1} dry soil)		Mg (mg kg^{-1} dry soil)		K (mg kg^{-1} dry soil)					
	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year	1-year	3-year
CT	17.9 (0.7)	16.8 (3.6)	5.71 (0.32)	5.96 (0.33)	4.42 (1.10)	9.74 (1.23)a	0.33 (0.07)	0.66 (0.10)	13.42 (0.52)a	14.70 (0.53)	179.7 (50.8)	211.5 (52.3)a	49.7 (15.6)	78.7 (27.4)	72.8 (7.0)	60.5 (14.9)	11.1 (4.4)	10.5 (2.7)	43.0 (6.9)	29.2 (12.2)
CTM	23.9 (2.1)	19.1 (3.2)	5.63 (0.29)	6.21 (0.45)	9.13 (3.39)	15.50 (2.96)b	0.60 (0.20)	0.95 (0.18)	15.15 (0.55)b	16.32 (1.46)	252.3 (37.7)	332.0 (78.1)b	65.5 (7.5)	105.7 (26.8)	69.7 (14.3)	60.9 (27.0)	15.8 (7.3)	15.0 (4.4)	31.2 (6.9)	25.3 (12.6)
NT	19.4 (4.6)	18.2 (1.9)	5.65 (0.19)	5.93 (0.17)	9.52 (2.22)	15.95 (2.49)b	0.64 (0.13)	1.12 (0.19)	14.88 (0.53)b	14.23 (0.22)	247.8 (83.7)	583.5 (170.7)c	58.4 (22.7)	105.8 (43.6)	72.1 (17.2)	93.2 (77.6)	20.4 (11.9)	37.2 (16.7)	21.5 (8.7)	12.3 (2.8)
NTM	20.0 (4.8)	23.4 (2.7)	5.53 (0.06)	5.84 (0.48)	11.36 (4.02)	15.92 (4.73)b	0.76 (0.28)	1.06 (0.31)	14.98 (0.35)b	15.01 (0.78)	217.8 (118.9)	537.5 (235.8)bc	51.0 (26.7)	128.7 (44.1)	69.3 (22.7)	88.3 (25.1)	19.3 (4.2)	27.4 (8.6)	34.3 (17.2)	18.5 (4.4)
ANOVA F -value																				
Tillage (T)	0.91	3.08	3.21	1.34	6.83*	5.48*	7.30*	7.47*	6.30*	5.35*	0.01	65.9***	1.51	6.60*	0.44	1.83	3.10	38.8***	1.26	8.71*
Mulch (M)	1.39	5.13*	0.00	0.21	2.85	4.03	2.77	1.16	6.71*	9.75*	0.01	1.10	0.01	6.54*	1.13	0.01	0.58	0.53	0.55	0.07
T×M	0.52	0.31	0.02	0.91	3.05	6.80*	2.48	2.84	9.29*	1.19	0.26	5.48*	0.04	0.04	1.61	0.01	1.55	4.52	2.66	2.39

The values in the upper portion of the table represent means, and the values in parentheses indicate standard deviations ($n=4$). Different letters within a row indicate a significant difference by Tukey's post hoc comparisons ($p<0.05$), when the ANOVA revealed a significant interaction effect

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

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

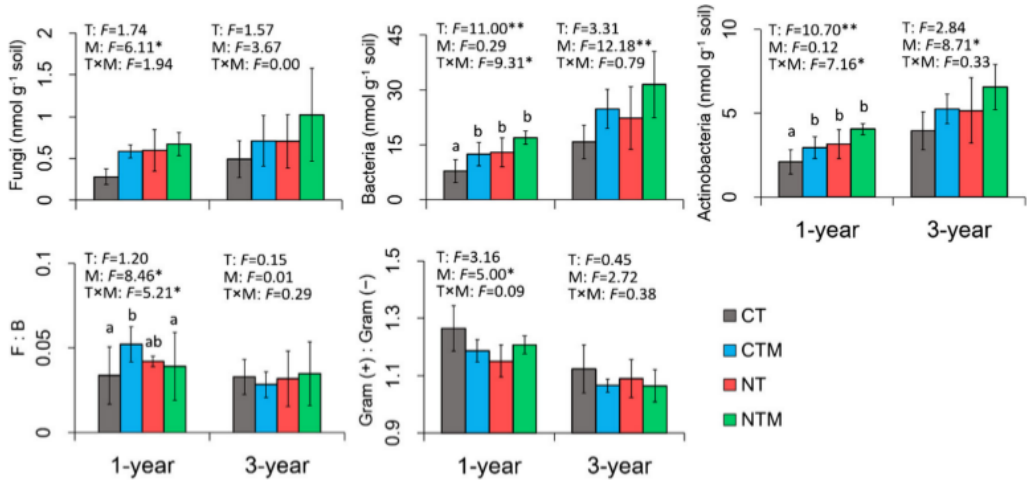

Fig. 1 The soil microbial PLFA concentrations of each treatment. The *error bars* represent standard deviations of means (n=4). The results of a two-way ANOVA with tillage (T) and bagasse mulching (M) in each year are shown. The *asterisks* indicate statistical significance: *p<0.05; **p<0.01; ***p<0.001. Different letters within the year indicate a significant difference by Tukey's post hoc comparisons (p<0.05). *CT* conventional tillage, *CTM* conventional tillage with bagasse mulching, *NT* no-tillage, *NTM* no-tillage with bagasse mulching

to 35 %. The majority of the taxonomic domains were Bacteria (98 %), followed by Eukaryota (1.2 %), and Archaea (0.8 %).

Taxonomic compositions of soil bacteria

Rarefaction curve analysis showed a decline in the slope of the gradient (Fig. S1), indicating that a reasonable number of species had been sampled. The ANOVA showed that there was an interaction effect of tillage×M treatment on alpha diversity in the first year (Fig. 2); alpha diversity was affected positively by the M treatment but only under the CT and by the NT treatment but only without the M treatment. Conversely, in the third year, there was a significant positive effect of the NT treatment on alpha diversity in the third year. The dominant bacterial phylum was Proteobacteria, which accounted for 35–49 % of the total soil metagenome sequences (Table 2). In the first year, the relative abundance of Proteobacteria was positively affected by the M treatment. In the third year, this phylum was positively affected by the NT and M treatments, but there was an interaction effect of tillage×M treatment (Table 2). According to the post hoc multiple comparison, the CT treatment had a significantly lower relative abundance of Proteobacteria than other treatments. The relative abundance of Gemmatimonadetes was increased by the NT treatment, whereas Thermotogae was decreased by the NT treatment in the first year. The relative abundance of Chlamydiae was increased by the NT treatment in the third year. The relative abundance of Deinococcus-Thermus was decreased by the M treatment in both years, whereas Fibrobacteres was increased by the M treatment in the third year. NMDS ordination showed there was a clear separation between the first-year and the third-year composition of bacterial phyla along the first axis (Fig. 3). Among treatments, the CT treatment was separated from the CTM and NTM treatments in the third year along the second axis, while there was no clear separation in the first year. The PERMANOVA analysis showed that there were significant effects of tillage and the M treatment on the taxonomic composition of bacterial phyla in the third year, while there were no significant differences in the first year (Table 4).

Soil functional profiles

ANOVA showed that the percentage of several metabolism-related sequences with respect to total sequences was significantly affected by treatments (Table 3). The percentage of

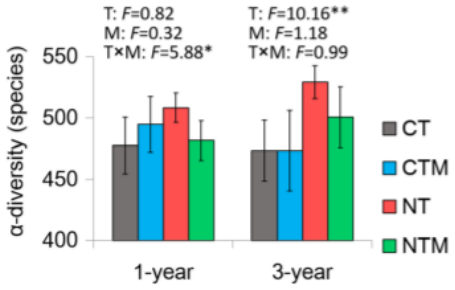

Fig. 2 The alpha diversity of each treatment. The *error bars* represent standard deviations of means (n=4). The results of a two-way ANOVA with tillage (T) and bagasse mulching (M) in each year are shown. The *asterisks* indicate statistical significance: *p<0.05; **p<0.01; ***p<0.001. *CT* conventional tillage, *CTM* conventional tillage with bagasse mulching, *NT* no-tillage, *NTM* no-tillage with bagasse mulching

Table 2 Relative abundances of bacterial phylum in each treatment soil

	% of total sequences								ANOVA					
	1-year				3-year				1-year			3-year		
	CT	CTM	NT	NTM	CT	CTM	NT	NTM	Till (T)	Much (M)	T x M	T	M	T x M
Acidobacteria	10.8 (3.40)	9.39 (2.51)	8.44 (1.48)	9.05 (2.59)	8.38 (0.87)	10.1 (2.21)	7.71 (1.94)	8.97 (1.65)	ns	ns	ns	ns	ns	ns
Actinobacteria	24.6 (6.35)	22.8 (3.70)	26.0 (4.29)	25.0 (5.67)	27.3 (3.22)	19.3 (2.36)	21.4 (6.55)	21.1 (6.93)	ns	ns	ns	ns	ns	ns
Aquificae	0.21 (0.03)	0.21 (0.01)	0.21 (0.02)	0.20 (0.02)	0.19 (0.01)	0.20 (0.01)	0.21 (0.03)	0.20 (0.05)	ns	ns	ns	ns	ns	ns
Bacteroidetes	2.17 (0.62)	2.33 (0.58)	2.11 (0.32)	2.09 (0.49)	2.18 (0.30)	2.47 (0.22)	3.06 (0.97)	3.01 (1.10)	ns	ns	ns	ns	ns	ns
Chlamydiae	0.10 (0.03)	0.10 (0.03)	0.10 (0.02)	0.11 (0.03)	0.09 (0.00)	0.10 (0.01)	0.14 (0.05)	0.17 (0.07)	ns	ns	ns	**	ns	ns
Chlorobi	0.42 (0.06)	0.42 (0.04)	0.40 (0.03)	0.40 (0.05)	0.39 (0.03)	0.44 (0.02)	0.45 (0.07)	0.41 (0.09)	ns	ns	ns	ns	ns	ns
Chloroflexi	5.41 (1.10)	4.56 (0.91)	4.79 (0.46)	4.66 (0.51)	3.93 (0.43)	3.26 (0.32)	3.53 (0.27)	2.96 (0.60)	ns	ns	ns	ns	*	ns
Chrysiogenetes	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.03 (0.00)	0.02 (0.01)	ns	ns	ns	ns	ns	ns
Cyanobacteria	2.39 (0.28)	2.25 (0.20)	2.30 (0.19)	2.24 (0.18)	2.43 (0.30)	2.33 (0.07)	2.30 (0.25)	2.13 (0.36)	ns	ns	ns	ns	ns	ns
Deferribacteres	0.08 (0.01)	0.08 (0.01)	0.08 (0.01)	0.08 (0.01)	0.07 (0.01)	0.07 (0.01)	0.08 (0.02)	0.08 (0.02)	ns	ns	ns	ns	ns	ns
Deinococcus-Thermus	0.85 (0.02)	0.78 (0.02)	0.82 (0.01)	0.79 (0.04)	0.86 (0.05)	0.78 (0.04)	0.81 (0.04)	0.73 (0.09)	ns	**	ns	ns	*	ns
Dictyoglomi	0.07 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.06 (0.01)	0.05 (0.01)	0.06 (0.01)	0.05 (0.01)	ns	ns	ns	ns	ns	ns
Elusimicrobia	0.02 (0.00)	0.03 (0.01)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.02 (0.01)	0.02 (0.01)	ns	ns	ns	ns	ns	ns
Fibrobacteres	0.015 (0.001)	0.019 (0.004)	0.014 (0.003)	0.015 (0.003)	0.015 (0.001)	0.018 (0.001)	0.016 (0.001)	0.021 (0.002)	ns	ns	ns	ns	***	ns
Firmicutes	6.40 (1.16)	6.18 (1.06)	6.14 (0.88)	6.00 (0.99)	5.15 (0.15)	4.65 (0.28)	5.10 (0.38)	4.66 (0.74)	ns	ns	ns	ns	ns	ns
Fusobacteria	0.087 (0.016)	0.091 (0.008)	0.083 (0.008)	0.077 (0.006)	0.072 (0.005)	0.077 (0.002)	0.086 (0.013)	0.070 (0.018)	ns	ns	ns	ns	ns	ns
Gemmatimonadetes	0.46 (0.03)	0.48 (0.03)	0.55 (0.05)	0.52 (0.06)	0.81 (0.14)	0.78 (0.12)	0.89 (0.09)	0.68 (0.13)	*	ns	ns	ns	ns	ns
Lentisphaerae	0.058 (0.015)	0.062 (0.002)	0.053 (0.007)	0.055 (0.006)	0.054 (0.006)	0.07 (0.003)	0.065 (0.011)	0.06 (0.013)	ns	ns	ns	ns	ns	ns
Nitrospirae	0.52 (0.08)	0.50 (0.07)	0.51 (0.09)	0.47 (0.06)	0.42 (0.04)	0.43 (0.05)	0.54 (0.13)	0.44 (0.11)	ns	ns	ns	ns	ns	ns
Planctomycetes	3.01 (0.43)	2.98 (0.34)	3.17 (0.37)	3.28 (0.58)	5.13 (1.16)	5.27 (0.32)	4.58 (0.60)	4.62 (0.63)	ns	ns	ns	ns	ns	ns
Poribacteria	0.060 (0.012)	0.059 (0.005)	0.063 (0.012)	0.060 (0.011)	0.052 (0.008)	0.052 (0.012)	0.065 (0.018)	0.053 (0.011)	ns	ns	ns	ns	ns	ns
Proteobacteria	38.2 (2.01)	42.4 (2.68)	40.2 (1.21)	40.9 (0.55)	38.7 (2.45)a	44.9 (1.53)b	44.5 (2.24)b	45.4 (2.34)b	ns	*	ns	*	**	*
Spirochaetes	0.23 (0.02)	0.23 (0.02)	0.22 (0.01)	0.22 (0.01)	0.22 (0.01)	0.23 (0.01)	0.24 (0.02)	0.22 (0.05)	ns	ns	ns	ns	ns	ns
Synergistetes	0.14 (0.01)	0.13 (0.00)	0.13 (0.01)	0.13 (0.01)	0.13 (0.01)	0.13 (0.01)	0.14 (0.01)	0.12 (0.02)	ns	ns	ns	ns	ns	ns
Tenericutes	0.033 (0.006)	0.034 (0.008)	0.031 (0.003)	0.028 (0.012)	0.025 (0.004)	0.024 (0.004)	0.027 (0.005)	0.027 (0.009)	ns	ns	ns	ns	ns	ns
Thermotogae	0.22 (0.02)	0.21 (0.02)	0.20 (0.001)	0.19 (0.01)	0.18 (0.02)	0.17 (0.02)	0.19 (0.02)	0.17 (0.03)	*	ns	ns	ns	ns	ns
Verrucomicrobia	2.53 (0.72)	2.75 (0.60)	2.54 (0.47)	2.58 (0.59)	2.37 (0.30)	3.36 (0.40)	2.96 (0.88)	2.83 (0.72)	ns	ns	ns	ns	ns	ns
unclassified	0.86 (0.08)	0.82 (0.08)	0.82 (0.05)	0.82 (0.02)	0.79 (0.03)	0.78 (0.03)	0.83 (0.05)	0.78 (0.13)	ns	ns	ns	ns	ns	ns

Values are means of replicate samples and parentheses indicate standard deviations (n=4). Different letters within rows of each year indicate a significant difference by Tukey's post hoc comparisons (p<0.05), when the ANOVA revealed a significant interaction effect

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

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

sequences related to carbohydrate metabolism was decreased by the NT treatment in both years. The M treatment also negatively affected these sequences in the third year. The percentage of sequences related to dormancy and sporulation was increased by the M treatment in the first year. The percentage of sequences related to secondary metabolism was increased by the NT treatment in the first year. The percentage of sequences related to stress response was increased by the NT and M treatments in the first year. There was an interaction effect of tillage×M treatment on the percentage of sequences related to photosynthesis in the first year; the CTM treatment had a significantly lower percentage of these sequences than the CT and NTM treatments according to the post hoc comparisons. In the third year, the percentage of sequences related to amino acids and derivatives was decreased by the M treatment, whereas the percentage of sequences related to miscellaneous, motility and chemotaxis, and sulfur metabolism was increased by the M treatment. The percentage of sequences related to DNA metabolism, miscellaneous, and sulfur metabolism was increased by the NT treatment, but the percentage of sequences related to motility and chemotaxis was decreased by the NT treatment in the third year. NMDS ordination showed that there was a separation between the first-year and the third-year functional composition along the second axis (Fig. 3). Among treatments, the CT treatment was slightly separated from other treatments in the third year along the first axis, while there was no clear separation in the first year. The PERMANOVA analysis showed that the functional composition was not significantly affected by treatments (Table 4). In contrast, the result of the NMDS ordination of subdivided carbohydrate metabolism genes showed a clear separation between CT and other treatments along the first axis (Fig. 4). The PERMANOVA analysis showed a significant effect of tillage on the composition of carbohydrate metabolism genes. According to the component loadings of individual categories, sequences assigned to the metabolism of all four known saccharide groups (monosaccharides, disaccharides, oligosaccharides, and polysaccharides), amino sugars, and glycoside hydrolases were located on negative axis 1, whereas central carbohydrate metabolism, fermentation, sugar alcohols, organic acids, CO_2 fixation, and one-carbon metabolism were located on positive axis 2.

Linking soil properties with microbial taxa and functional categories

The concentrations of bacterial and fungal PLFAs had a significant positive correlation with total soil C and N in both years, according to a Spearman's correlation analysis (Table 5).

The links between the metagenomic profiles and the measured soil parameters were also investigated by Spearman's correlations (Table 5). First, alpha diversity had strong negative correlation with soluble Al content in both years. The relative abundance of Proteobacteria, which were significantly increased by the NT and M treatments, was positively correlated with total soil C and N and C/N ratio and negatively correlated with soluble Al content in both years. Chlamydiae, which were significantly increased by the NT treatment in the third year, were positively correlated with exchangeable Ca and available P content in the third year. Deinococcus-Thermus, which were decreased by the M treatment, were negatively correlated with total soil C and N and available P content in the first year.

Spearman's correlations between the percentages of sequences related to microbial metabolisms and the values for soil parameters were also calculated (Table 6). The percentage of sequences related to carbohydrate metabolisms, which was significantly higher in the CT treatment, was negatively correlated with total soil C and N and negatively correlated with soluble Al content in both years. The percentage of sequences related to amino acids and derivatives, which was decreased by the M treatment in the third year, was also negatively correlated with soil C/N ratio and exchangeable Mg content in the third year. The percentage of sequences related to dormancy, which was

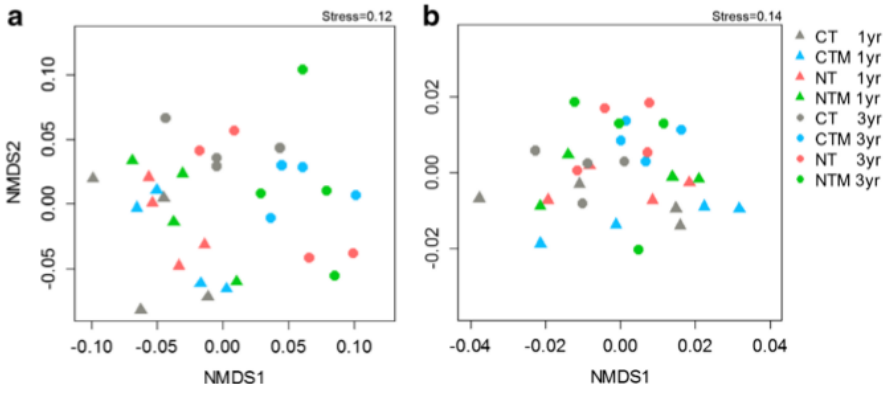

Fig. 3 The NMDS of **a** taxonomic (bacteria at the phylum level) and **b** functional (SEED subsystem level 1) profiles in each treatment. 2D stress is shown in the upper right of each figure. *CT* conventional tillage, *CTM* conventional tillage with bagasse mulching, *NT* no-tillage, *NTM* no-tillage with bagasse mulching

Table 3 Percentage of functional categories (SEED subsystem level 1) in each treatment soil

	% of total sequences								ANOVA					
	1-year				3-year				1-year			3-year		
	CT	CTM	NT	NTM	CT	CTM	NT	NTM	Till (T)	Much (M)	T x M	T	M	T x M
Amino Acids and Derivatives	8.89 (0.38)	8.88 (0.36)	8.98 (0.25)	8.84 (0.35)	8.98 (0.09)	8.84 (0.09)	8.94 (0.12)	8.81 (0.17)	ns	ns	ns	ns	*	ns
Carbohydrates metabolism	11.7 (0.03)	11.6 (0.13)	11.5 (0.02)	11.5 (0.15)	11.7 (0.16)	11.3 (0.14)	11.3 (0.16)	11.2 (0.30)	*	ns	ns	**	*	ns
Cell Division and Cell Cycle	1.33 (0.07)	1.30 (0.05)	1.32 (0.07)	1.33 (0.08)	1.31 (0.06)	1.29 (0.04)	1.28 (0.05)	1.29 (0.06)	ns	ns	ns	ns	ns	ns
Cell Wall and Capsule	3.28 (0.22)	3.30 (0.19)	3.27 (0.21)	3.25 (0.21)	3.32 (0.02)	3.43 (0.08)	3.39 (0.16)	3.44 (0.12)	ns	ns	ns	ns	ns	ns
Clustering-based subsystems	15.2 (0.12)	15.1 (0.10)	15.2 (0.14)	15.2 (0.20)	15.3 (0.13)	15.1 (0.08)	15.3 (0.15)	15.2 (0.12)	ns	ns	ns	ns	ns	ns
Cofactors, Vitamins, Prosthetic Groups, Pigments	6.32 (0.23)	6.27 (0.21)	6.39 (0.25)	6.33 (0.20)	6.53 (0.09)	6.39 (0.19)	6.47 (0.07)	6.54 (0.10)	ns	ns	ns	ns	ns	ns
DNA Metabolism	3.67 (0.05)	3.67 (0.04)	3.67 (0.08)	3.62 (0.02)	3.66 (0.05)	3.64 (0.03)	3.81 (0.09)	3.73 (0.07)	ns	ns	ns	***	ns	ns
Dormancy and Sporulation	0.19 (0.02)	0.22 (0.02)	0.21 (0.01)	0.22 (0.01)	0.19 (0.02)	0.20 (0.01)	0.20 (0.01)	0.19 (0.01)	ns	*	ns	ns	ns	ns
Fatty Acids, Lipids, and Isoprenoids	3.24 (0.16)	3.16 (0.10)	3.23 (0.09)	3.30 (0.13)	3.36 (0.12)	3.26 (0.07)	3.28 (0.11)	3.26 (0.17)	ns	ns	ns	ns	ns	ns
Iron acquisition and metabolism	0.46 (0.07)	0.48 (0.05)	0.49 (0.03)	0.47 (0.06)	0.44 (0.04)	0.51 (0.03)	0.52 (0.05)	0.51 (0.06)	ns	ns	ns	ns	ns	ns
Membrane Transport	3.32 (0.16)	3.35 (0.22)	3.33 (0.20)	3.30 (0.18)	3.19 (0.13)	3.31 (0.08)	3.30 (0.07)	3.23 (0.14)	ns	ns	ns	ns	ns	ns
Metabolism of Aromatic Compounds	2.05 (0.07)	2.03 (0.07)	2.08 (0.08)	2.10 (0.08)	2.16 (0.04)	2.12 (0.08)	2.05 (0.04)	2.13 (0.15)	ns	ns	ns	ns	ns	ns
Miscellaneous	7.72 (0.08)	7.63 (0.11)	7.58 (0.09)	7.67 (0.09)	7.73 (0.06)	7.84 (0.05)	7.85 (0.09)	7.94 (0.09)	ns	ns	ns	*	*	ns
Motility and Chemotaxis	0.92 (0.07)	1.01 (0.08)	0.97 (0.05)	0.96 (0.06)	0.99 (0.05)	1.07 (0.04)	0.96 (0.01)	0.99 (0.03)	ns	ns	ns	**	*	ns
Nitrogen Metabolism	1.21 (0.05)	1.28 (0.06)	1.22 (0.03)	1.22 (0.07)	1.18 (0.03)	1.25 (0.07)	1.27 (0.04)	1.24 (0.07)	ns	ns	ns	ns	ns	ns
Nucleosides and Nucleotides	2.88 (0.07)	2.82 (0.16)	2.88 (0.12)	2.85 (0.15)	2.88 (0.10)	2.86 (0.04)	2.86 (0.05)	2.88 (0.06)	ns	ns	ns	ns	ns	ns
Phages, Prophages, Transposable elements, Plasmids	1.32 (0.05)	1.30 (0.05)	1.32 (0.05)	1.30 (0.04)	1.25 (0.05)	1.25 (0.04)	1.23 (0.02)	1.27 (0.05)	ns	ns	ns	ns	ns	ns
Phosphorus Metabolism	0.90 (0.05)	0.91 (0.05)	0.92 (0.02)	0.89 (0.02)	0.88 (0.06)	0.92 (0.02)	0.90 (0.04)	0.92 (0.04)	ns	ns	ns	ns	ns	ns
Photosynthesis	0.13 (0.01)a	0.11 (0.01)b	0.13 (0.01)ab	0.14 (0.03)a	0.14 (0.00)	0.14 (0.01)	0.13 (0.02)	0.14 (0.01)	ns	ns	*	ns	ns	ns
Potassium metabolism	0.51 (0.04)	0.53 (0.06)	0.52 (0.03)	0.55 (0.03)	0.51 (0.02)	0.51 (0.03)	0.48 (0.02)	0.49 (0.03)	ns	ns	ns	ns	ns	ns
Protein Metabolism	7.98 (0.24)	8.10 (0.13)	8.04 (0.19)	8.04 (0.27)	7.84 (0.13)	7.71 (0.12)	7.82 (0.09)	7.76 (0.22)	ns	ns	ns	ns	ns	ns
RNA Metabolism	3.72 (0.11)	3.77 (0.01)	3.69 (0.13)	3.69 (0.04)	3.62 (0.05)	3.67 (0.02)	3.71 (0.05)	3.71 (0.17)	ns	ns	ns	ns	ns	ns
Regulation and Cell signaling	1.42 (0.07)	1.45 (0.05)	1.42 (0.08)	1.44 (0.02)	1.39 (0.08)	1.46 (0.03)	1.42 (0.03)	1.41 (0.03)	ns	ns	ns	ns	ns	ns
Respiration	4.10 (0.10)	4.09 (0.10)	4.05 (0.09)	4.11 (0.16)	3.99 (0.07)	3.98 (0.12)	3.90 (0.06)	3.95 (0.09)	ns	ns	ns	ns	ns	ns
Secondary Metabolism	0.46 (0.01)	0.46 (0.02)	0.49 (0.02)	0.50 (0.02)	0.49 (0.02)	0.52 (0.02)	0.52 (0.05)	0.52 (0.04)	**	ns	ns	ns	ns	ns
Stress Response	2.51 (0.02)	2.60 (0.06)	2.61 (0.04)	2.62 (0.05)	2.59 (0.03)	2.62 (0.07)	2.57 (0.03)	2.59 (0.10)	*	*	ns	ns	ns	ns
Sulfur Metabolism	1.30 (0.04)	1.31 (0.04)	1.33 (0.07)	1.36 (0.07)	1.31 (0.04)	1.40 (0.02)	1.38 (0.04)	1.41 (0.03)	ns	ns	ns	*	**	ns
Virulence, Disease and Defense	3.21 (0.30)	3.27 (0.26)	3.18 (0.20)	3.23 (0.15)	3.13 (0.12)	3.35 (0.23)	3.22 (0.18)	3.28 (0.17)	ns	ns	ns	ns	ns	ns

Values are means of replicate sample and parentheses indicate standard deviation (n=4). Different letters within rows of each year indicate a significant difference by Tukey's post hoc comparisons (p<0.05), when the ANOVA revealed a significant interaction effect

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

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

Table 4 PERMANOVA results for differences in taxonomic and functional compositions

	1-year						3-year					
	Df	Sum of squares	Mean of squares	F Model	R2	*p* value	Df	Sum of squares	Mean of squares	F Model	R2	*p* value
Taxonomy (bacteria at the phylum level)												
Till (T)	1	0.00244	0.00244	1.04372	0.07341	0.364	1	0.00510	0.00510	2.3117	0.12290	0.042*
Mulch (M)	1	0.00205	0.00204	0.87444	0.06151	0.462	1	0.00672	0.00672	3.0424	0.16175	0.028*
T×M	1	0.00070	0.00070	0.29923	0.02105	0.976	1	0.00321	0.00321	1.4556	0.07739	0.175
Function (SEED subsystem level 1)												
T	1	0.00032	0.00032	0.8728	0.06131	0.379	1	0.00038	0.00038	1.7583	0.10661	0.078
M	1	0.00027	0.00027	0.72613	0.05101	0.510	1	0.00031	0.00031	1.4529	0.08809	0.172
T×M	1	0.00023	0.00023	0.63658	0.04472	0.626	1	0.00027	0.00027	1.2816	0.07771	0.247

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

increased by the M treatment in the first year, was positively correlated with the soil C/N ratio in the first year. The percentage of sequences related to DNA metabolism, which was higher in the NT treatment in the third year, was positively correlated with exchangeable Ca and available P content and negatively correlated with soluble Al content in the third year. The percentage of sequences related to secondary metabolism, which was higher in the NT treatment in the first year, was positively correlated with total soil C and N content in the first year. The percentage of sequences related to stress response, which was increased by NT and M treatment in the first year, was positively correlated with total soil C and N content, soil C/N ratio, and available P content and negatively correlated with soluble Al content in the first year. The percentage of sequences related to sulfur metabolism, which was increased by the NT and M treatments in the third year, was positively correlated with total soil C and N, exchangeable Ca, and available P content in the third year.

Discussion

Effects of NT and bagasse mulching on soil microbial communities

In many cases, the conversion from CT to NT practices increases microbial biomass in surface soil (e.g., Wardle 1995; Kladivko 2001; Silvia et al. 2014). Van Veen and Paul (1978) reported that microbial biomass decreased with an increase in tillage disturbance because of lower organic matter inputs to the soil. Soils under NT have higher crop residues on the surface and therefore a greater organic matter content (Feng et al. 2003), which in turn increases the soil microbial biomass because of the higher amount of substrate available for microbial growth (Kandeler et al. 1999).

We expected that bagasse would increase the microbial biomass, especially the fungal biomass in the soil, because bagasse has a higher C/N ratio than soil and sugarcane leaf

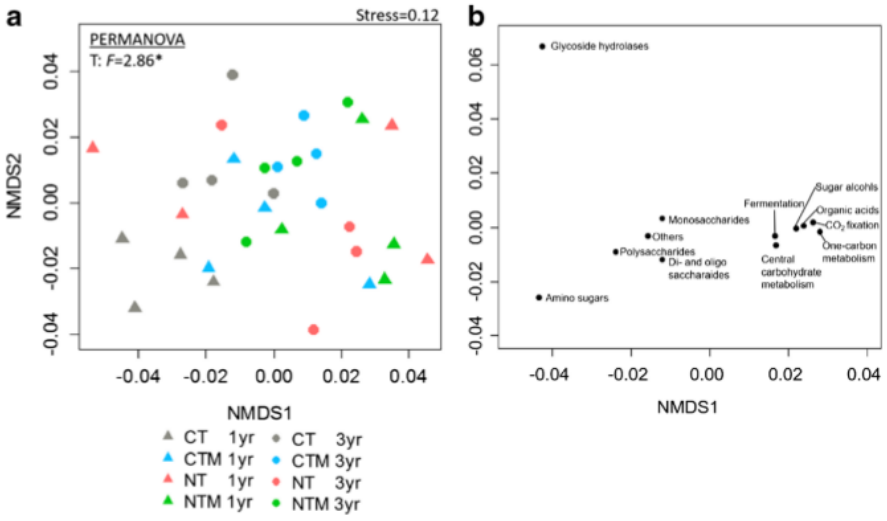

Fig. 4 The NMDS of **a** compositions of subdivided carbohydrate metabolisms and **b** component loadings of individual categories (SEED subsystem level 2). 2D stress is shown in the upper right of the figure. The result of the permutational multivariable analysis of variance (PERMANOVA) for differences in compositions among tillage (T), bagasse mulching, experimental year, and these interactions is also given (significant effects only: *p<0.05). *CT* conventional tillage, *CTM* conventional tillage with bagasse mulching, *NT* no-tillage, *NTM* no-tillage with bagasse mulching

Table 5 Spearman's correlation values between soil parameters and microbial PLFAs, diversity, and relative abundance of each bacterial phylum in each year of the experiment

	Moisture	pH	C	N	C/N	Available				
						Ca	Mg	K	P	Al
Fungal PLFA	−0.0/0.3	0.1/−0.0	**0.5**/0.4	**0.5**/0.4	0.4/0.0	0.1/0.3	−0.1/0.2	−0.2/−0.2	**0.6**/0.1	−0.4/−0.3
Bacterial PLFA	0.0/0.4	−0.1/−0.2	**0.6**/**0.6**	**0.6**/**0.5**	0.4/0.1	0.1/0.4	−0.1/0.4	−0.0/−0.1	**0.6**/0.3	−0.3/−0.2
Gram-positive bacterial PLFA	0.1/0.5	−0.2/−0.2	**0.6**/**0.7**	**0.6**/**0.6**	0.4/0.1	0.1/0.4	−0.0/0.4	0.0/−0.1	**0.6**/0.3	−0.2/−0.3
Gram-negative bacterial PLFA	0.0/0.4	−0.1/−0.2	**0.6**/**0.6**	**0.6**/**0.5**	**0.5**/0.1	0.1/0.4	−0.1/0.4	−0.1/−0.1	**0.7**/0.3	−0.3/−0.2
Actinobacterial PLFA	0.0/0.4	−0.1/−0.2	**0.6**/**0.6**	**0.6**/**0.5**	0.4/0.0	0.1/0.4	−0.1/0.4	−0.0/−0.0	**0.6**/0.3	−0.3/−0.2
F:B	0.2/0.0	0.2/0.3	**0.5**/−0.1	**0.5**/0.1	**0.5**/−0.3	0.1/-0.0	0.1/−0.0	−0.4/−0.0	0.3/−0.2	−0.3/−0.3
Gram-positive/Gram-negative	0.2/−0.0	−0.3/−0.1	−0.4/−0.3	−0.4/−0.3	−0.4/0.1	−0.2/−0.4	−0.1/−0.4	0.3/0.1	**−0.6**/−0.4	0.4/0.3
α-diversity	0.2/0.2	**0.5**/0.1	0.3/**0.6**	0.3/**0.7**	**0.5**/−0.3	0.4/**0.8**	0.4/**0.5**	0.0/0.2	0.1/**0.7**	**−0.7**/**−0.9**
Acidobacteria	−0.2/−0.3	−0.3/-0.2	−0.3/−0.1	−0.3/−0.2	−0.2/0.4	**−0.5**/−0.2	**−0.5**/−0.0	−0.2/−0.0	−0.2/0.0	0.4/0.4
Actinobacteria	−0.2/0.0	−0.2/0.2	0.2/−0.4	0.2/−0.4	−0.1/−0.2	0.3/−0.4	0.4/−0.4	−0.0/0.1	0.0/−0.4	−0.3/0.1
Aquificae	−0.1/−0.1	−0.3/0.3	−0.1/0.1	−0.1/0.1	0.1/0.2	−0.4/0.1	−0.4/0.1	−0.1/−0.0	0.1/0.2	0.1/0.0
Bacteroidetes	0.2/0.1	−0.1/0.2	0.2/0.4	−0.2/0.4	0.2/-0.0	−0.2/**0.7**	−0.2/**0.7**	−0.1/0.1	−0.1/**0.6**	0.1/−0.4
Chlamydiae	0.2/0.3	−0.1/-0.1	−0.1/0.3	−0.1/0.2	0.2/0.0	−0.2/**0.6**	−0.3/0.4	0.0/0.3	0.1/**0.6**	0.2/−0.1
Chlorobi	0.2/0.0	−0.2/0.2	−0.3/0.4	−0.3/0.3	0.0/0.2	−0.3/0.3	−0.3/0.4	0.0/-0.1	−0.1/0.3	0.2/−0.1
Chloroflexi	−0.3/**−0.5**	−0.1/0.3	−0.4/**−0.5**	−0.3/−0.4	−0.3/0.0	**−0.5**/−0.3	**−0.5**/−0.3	−0.1/0.0	−0.1/−0.2	0.4/0.1
Chrysiogenetes	0.3/−0.2	−0.4/0.3	0.4/0.2	0.3/0.3	0.4/0.1	0.0/0.0	0.1/0.0	−0.3/−0.2	0.1/-0.1	−0.4/−0.1
Cyanobacteria	−0.1/−0.4	−0.2/**0.5**	−0.4/−0.1	−0.4/−0.1	−0.1/−0.1	−0.4/0.0	−0.4/0.1	−0.1/0.0	−0.1/−0.1	0.2/0.0
Deferribacteres	0.0/−0.1	−0.4/0.3	−0.2/0.4	−0.2/0.4	−0.2/0.0	−0.4/**0.5**	−0.4/0.3	0.0/0.0	0.0/0.4	0.0/-0.3
Deinococcus-Thermus	−0.2/−0.4	0.3/0.2	**−0.5**/−0.3	**−0.5**/−0.2	−0.4/0.0	0.1/−0.3	0.2/−0.3	0.3/−0.1	**−0.6**/−0.3	−0.1/−0.1
Dictyoglomi	−0.4/**−0.6**	−0.2/0.2	−0.4/−0.3	−0.4/−0.4	−0.4/0.2	−0.4/−0.3	**−0.5**/−0.3	−0.1/−0.2	0.0/−0.2	0.4/0.4
Elusimicrobia	0.4/−0.3	−0.1/0.2	−0.2/0.1	−0.2/0.0	0.2/0.2	0.3/0.0	0.1/0.1	0.1/−0.1	0.0/0.0	0.0/0.2
Fibrobacteres	0.3/**0.6**	0.1/0.0	0.0/0.2	0.0/0.2	**0.6**/0.3	0.1/0.3	0.1/0.4	0.1/0.4	0.2/0.2	0.0/-0.1
Firmicutes	0.0/-0.3	−0.2/0.3	−0.3/−0.4	−0.2/−0.3	0.1/0.0	−0.3/−0.2	−0.3/−0.2	0.0/0.1	−0.1/0.0	0.3/0.2
Fusobacteria	0.1/−0.1	0.0/0.4	−0.2/0.4	−0.2/0.4	0.0/0.0	−0.4/0.3	−0.2/0.1	−0.1/−0.3	−0.1/0.3	0.1/−0.3
Gemmatimonadetes	0.1/-0.1	−0.2/0.3	0.1/0.4	0.1/**0.5**	0.1/−0.2	0.2/0.4	0.2/0.2	0.3/−0.1	0.2/0.2	−0.4/**−0.6**
Lentisphaerae	0.1/-0.1	−0.1/0.2	0.0/**0.5**	0.0/0.4	0.2/0.2	−0.3/0.3	−0.4/0.3	−0.1/-0.2	0.2/0.3	0.2/−0.1
Nitrospirae	−0.1/0.1	−0.2/0.3	−0.2/0.4	−0.2/0.4	0.1/0.0	**−0.5**/**0.6**	**−0.5**/**0.5**	−0.3/0.2	−0.1/**0.6**	0.1/-0.4
Planctomycetes	0.2/−0.4	−0.1/0.1	−0.2/0.1	−0.2/0.0	0.1/0.2	−0.1/−0.2	−0.2/0.1	0.1/-0.4	0.0/−0.3	0.0/0.3
Poribacteria	0.1/0.0	−0.4/0.2	−0.1/0.3	−0.1/0.3	0.2/0.1	−0.3/**0.5**	−0.3/0.4	−0.2/0.1	0.1/**0.5**	−0.1/−0.3
Proteobacteria	0.4/0.4	0.0/0.0	**0.5**/**0.8**	**0.5**/**0.7**	**0.6**/0.1	0.2/**0.7**	0.2/**0.6**	−0.1/0.1	0.3/**0.5**	**−0.6**/**−0.5**
Spirochaetes	0.0/−0.2	0.1/0.2	**−0.5**/0.3	**−0.5**/0.3	−0.1/0.1	−0.3/0.3	−0.2/0.2	0.1/0.0	−0.2/0.3	0.3/0.0
Synergistetes	0.3/-0.4	−0.2/0.2	**−0.5**/−0.1	**−0.5**/−0.2	−0.2/0.1	0.2/0.1	0.4/−0.1	0.3/0.1	**−0.6**/0.3	0.0/0.1
Tenericutes	−0.3/−0.2	0.2/0.4	0.1/−0.1	0.1/0.0	−0.1/−0.1	0.0/0.3	0.0/0.4	0.1/0.2	0.2/0.3	0.3/−0.1
Thermotogae	−0.1/−0.3	0.0/0.1	**−0.5**/0.1	−0.4/0.0	−0.2/0.2	−0.2/0.0	−0.2/−0.2	−0.1/−0.2	−0.2/0.2	0.3/0.0
Verrucomicrobia	0.1/−0.1	−0.1/0.2	−0.2/0.4	−0.2/0.3	0.1/0.2	−0.4/0.3	−0.4/0.4	−0.2/−0.2	0.1/0.2	0.1/−0.1
unclassified	−0.1/−0.2	−0.3/0.0	−0.3/0.2	−0.3/0.1	−0.1/0.1	**−0.5**/0.4	**−0.5**/0.2	−0.1/−0.1	−0.1/**0.5**	0.3/0.0

Slash separates the results of correlation values in each year. Significant correlation values are shown in bold font

litter (Miura et al. 2013) and is a lignin-rich substrate (Chandel et al. 2012). In the first year of the experiment, soils under CTM had a significantly higher F/B ratio than other treatments, as expected. However, there was no significant effect of the M treatment on soil fungal PLFA in the third year. In contrast, total bacterial PLFAs was significantly higher in the M treatment. In addition, the Gram-positive/Gram-negative bacteria ratio was slightly lower in the M treatment. Gram-negative bacteria are reported to use a fresher C source, derived from plant biomass, while Gram-positive bacteria are reported to use a more aged C source, derived from SOM (Kramer and Gleixner 2006; Potthast et al. 2012). The lower Gram-positive/Gram-negative bacteria ratio in the M-treated soils than in soils under the other treatments suggests that the M-treated soils had a more easily degradable substrate. Approximately 80 % of bagasse dry mass decomposed in

Table 6 Spearman's correlation values between soil parameters and functional categories (SEED subsystem level 1) in each year of the experiment

	Moisture	pH	C	N	C/N	Available				
						Ca	Mg	K	P	Al
Amino Acids and Derivatives	−0.1/−0.4	0.4/−0.3	0.2/−0.3	0.2/−0.2	0.0/**−0.5**	0.4/−0.4	0.4/**−0.7**	0.1/−0.2	0.0/−0.3	−0.2/0.1
Carbohydrates metabolism	−0.3/**−0.5**	0.2/−0.2	**−0.5**/**−0.8**	**−0.5**/**−0.7**	−0.4/−0.2	0.0/**−0.9**	−0.1/**−0.9**	0.2/−0.3	−0.2/**−0.7**	**0.5**/**0.7**
Cell Division and Cell Cycle	−0.4/−0.2	0.2/0.1	0.0/0.0	−0.1/0.0	−0.2/0.3	0.0/-0.1	0.0/0.0	0.2/−0.4	0.3/−0.3	0.0/0.0
Cell Wall and Capsule	0.2/0.1	−0.2/0.2	−0.3/0.3	−0.3/0.2	0.0/0.4	−0.3/0.3	−0.3/0.4	−0.1/0.1	−0.3/0.3	0.2/0.1
Clustering-based subsystems	0.1/0.2	−0.2/−0.1	−0.3/0.0	−0.3/0.0	−0.2/0.1	0.1/0.2	0.1/0.2	−0.2/0.2	−0.4/0.3	−0.3/−0.2
Cofactors, Vitamins, Prosthetic Groups, Pigments	0.2/0.3	0.3/−0.3	0.1/−0.1	0.1/-0.1	0.1/0.1	**0.7**/−0.1	**0.7**/−0.2	0.3/0.3	−0.1/0.1	−0.4/0.0
DNA Metabolism	0.0/0.2	**0.6**/0.0	−0.2/0.4	−0.1/0.4	−0.3/−0.2	0.0/**0.7**	0.1/0.4	0.4/0.1	0.0/**0.7**	0.2/**−0.6**
Dormancy and Sporulation	0.3/−0.1	0.2/−0.1	0.4/0.0	0.4/0.0	**0.7**/−0.4	0.2/−0.1	0.1/−0.1	−0.1/0.3	**0.5**/0.0	0.0/−0.1
Fatty Acids, Lipids, and Isoprenoids	0.0/0.1	0.0/−0.2	0.1/−0.2	0.1/−0.1	0.0/−0.2	0.3/−0.2	0.3/−0.3	0.0/0.0	0.0/−0.1	−0.3/−0.1
Iron acquisition and metabolism	−0.1/0.3	−0.1/0.0	0.0/**0.5**	0.0/**0.6**	0.0/−0.3	**−0.5**/**0.7**	**−0.5**/**0.6**	−0.2/0.1	0.1/0.4	0.2/**−0.6**
Membrane Transport	−0.1/0.1	−0.4/**0.5**	0.0/0.4	0.0/**0.5**	−0.1/0.0	**−0.6**/**0.6**	**−0.5**/**0.7**	−0.2/0.3	0.1/0.1	−0.1/**−0.6**
Metabolism of Aromatic Compounds	0.1/0.1	−0.1/0.1	0.3/−0.3	0.3/−0.3	0.2/−0.2	0.4/−0.3	0.4/−0.2	0.1/0.1	0.0/**−0.5**	**−0.5**/0.1
Miscellaneous	0.1/**0.6**	0.2/−0.1	−0.4/0.4	−0.4/0.4	−0.1/−0.2	0.3/**0.7**	0.3/**0.5**	0.0/**0.5**	−0.3/**0.6**	0.2/−0.4
Motility and Chemotaxis	0.3/−0.1	**−0.5**/0.4	0.1/0.1	0.1/0.0	0.3/0.4	−0.3/−0.2	−0.2/0.2	−0.3/−0.2	0.1/−0.4	−0.2/0.1
Nitrogen Metabolism	0.2/0.2	0.0/0.1	0.3/**0.5**	0.3/**0.5**	0.1/−0.2	0.1/**0.5**	0.2/0.3	−0.1/0.3	0.2/**0.5**	−0.3/**-0.5**
Nucleosides and Nucleotides	0.2/0.0	0.2/−0.4	−0.1/0.1	−0.1/0.0	0.0/0.2	**0.7**/0.0	**0.7**/0.1	0.4/−0.3	−0.1/0.2	−0.3/0.2
Phages, Prophages, Transposable elements, Plasmids	−0.4/−0.1	−0.4/−0.1	0.0/−0.2	0.0/−0.4	−0.1/0.2	**−0.5**/−0.3	**−0.5**/−0.3	0.1/0.0	0.2/−0.1	0.4/**0.6**
Phosphorus Metabolism	0.0/0.4	0.3/**0.5**	−0.1/0.2	−0.1/0.2	−0.1/0.3	−0.2/0.4	−0.1/**0.7**	−0.3/0.1	−0.4/0.3	−0.1/−0.3
Photosynthesis	−0.1/−0.2	0.0/0.2	−0.1/−0.3	−0.1/−0.3	−0.3/0.0	0.3/−0.4	0.4/**−0.5**	0.4/−0.3	−0.1/−0.4	0.0/0.2
Potassium metabolism	0.1/−0.1	**−0.6**/0.1	0.3/−0.3	0.3/−0.3	0.2/0.2	−0.4/−0.4	−0.3/−0.2	−0.1/−0.3	0.1/**−0.5**	0.2/0.3
Protein Metabolism	−0.2/0.0	−0.1/0.1	0.3/0.0	0.3/0.1	0.4/−0.1	−0.3/−0.1	−0.4/0.0	−0.4/0.1	**0.5**/0.0	0.2/−0.1
RNA Metabolism	0.0/-0.1	0.2/0.3	−0.1/**0.5**	−0.1/**0.5**	−0.1/0.0	−0.1/**0.6**	−0.1/**0.5**	0.3/0.2	0.3/**0.5**	0.4/**−0.6**
Regulation and Cell signaling	0.3/−0.2	0.0/0.3	−0.2/0.1	−0.2/0.1	0.0/0.1	−0.2/0.1	−0.3/0.2	0.1/0.1	−0.3/−0.1	0.2/0.1
Respiration	−0.3/-0.4	−0.3/0.1	0.0/**−0.5**	0.0/**−0.6**	−0.2/0.4	**−0.6**/**−0.5**	**−0.7**/−0.3	−0.2/−0.2	0.1/−0.2	0.3/**0.7**
Secondary Metabolism	0.0/0.0	−0.2/0.0	**0.6**/0.3	**0.6**/0.3	0.4/-0.2	0.0/0.4	0.1/0.2	−0.1/−0.1	0.4/0.2	−0.4/−0.1
Stress Response	0.2/0.2	0.1/−0.3	**0.7**/0.2	**0.7**/0.3	**0.5**/−0.1	0.3/0.0	0.3/0.0	−0.1/−0.1	**0.6**/-0.3	**−0.6**/−0.2
Sulfur Metabolism	0.1/**0.5**	−0.1/−0.3	0.3/**0.7**	0.3/**0.6**	0.4/0.2	−0.1/**0.5**	−0.2/0.4	−0.3/0.2	0.0/**0.5**	−0.4/−0.2
Virulence, Disease and Defense	0.1/−0.2	−0.2/0.2	−0.2/0.4	−0.2/0.3	0.0/0.2	−0.4/0.2	−0.4/0.2	0.0/**−0.5**	−0.1/0.1	0.4/0.0

Slash separates the results of correlation values in each year. Significant correlation values are shown in bold font

the sampling period (data not shown). Bagasse quality must have gradually changed from recalcitrant to degradable, thus causing a shift to a Gram-negative bacterial dominant soil.

The shotgun metagenome sequencing results indicated that the alpha diversity of soil microbial communities was increased by the NT treatment, which agrees with several other studies (Ceja-Navarro et al. 2009; Dorr de Quadros et al. 2012; Sengupta and Dick 2015) that have shown that soils under the NT system had a higher bacterial diversity than those under the CT system. In this study, alpha diversity had a significant negative correlation with soluble Al content. Principally, Al is toxic since it competes with Fe and Mg, binding to DNA, membranes, or cell walls (Piña and Cervantes 1996). Therefore, NT soils with a low soluble Al content might provide a favorable environment for a larger number of microbial species than CT soils with a high soluble Al content.

This study indicated that the NT and M treatments increased the relative abundance of Proteobacteria. Fierer et al. (2007) and Park et al. (2015) reported that Proteobacteria are more abundant in soils with higher C availability, which supports the results of our study. Our results also indicated that the M treatment had a higher relative abundance of Fibrobacteres. Many species of Fibrobacteres are recognized as major bacterial degraders of lignocellulosic material (Ransom-Jones et al. 2012). Bagasse substrate, which has high lignin content, is likely to be the reason for the increased abundance of this phylum. In contrast, the M treatment decreased the relative abundance of Chloroflexi and Deinococcus-Thermus. These phyla contain a large number of thermophilic species (Lau et al. 2009; Tian and Hua 2010). Extremophiles, such as thermophilic bacteria, can only live in special environments with no competition. Therefore, their

adaptability to other environments can be low (Pietra 2002). When bagasse application enhances the percentage of microbial species that prefer the bagasse substrate, extremophiles may not be able to withstand the interspecific competition.

Chlamydiae, which are known as pathogens of animals and humans, were more abundant in the NT treatment. However, their function in the soil environment is not known.

Effects of NT and bagasse mulching on soil functional profiles

The PERMANOVA analysis showed that the effect of different agricultural systems on the soil functional composition was not significant. Together with previous studies (Pan et al. 2014; Souza et al. 2015; Wood et al. 2015), our results suggested that the functional profiles of soil microbial communities are more resistant to agricultural managements than to community composition. However, we showed that some metabolism genes, which should have an important role in soil processes, were significantly affected by different agricultural practices. Compared with the NT and M treatments, the CT treatment had a higher percentage of sequences related to carbohydrate metabolism, and there was a negative correlation between the percentage of these sequences and the soil C content. This is a similar finding to that of Souza et al. (2015). In addition, our results from the NMDS ordination of subdivided carbohydrate metabolisms indicate that more sequences related to the metabolism of all four saccharide groups were present in the CT treatment than in other treatments (Fig. 4). A fraction of soil polysaccharides in soil organic C is subject to long-term stabilization (Kiem and Kögel-Knabner 2003). One of the reasons is that soil polysaccharide is adsorbed by clay, and this complex is recalcitrant to microbial degradation (Cheshire 1977). The recalcitrant C metabolism genes should be enriched in soils under the CT treatment, since the labile C was lower than in the NT and M treatments. In addition, sequences of glycoside hydrolases that typically cleave oligo/polysaccharides into small monosaccharides were also presented more in CT soils, suggesting that microorganisms in CT soils allocate a substantial amount of energy to utilize sugar compounds.

In contrast to the CT treatment, soils under the NT treatment had higher percentage of sequences related to DNA metabolism. There were positive correlations between DNA metabolism and exchangeable Ca and available P contents. This supports several studies that suggest that the higher nutrient level may enhance microbial reproduction (Pan et al. 2014; Smith et al. 2012). Zhang et al. (2014) indicated that the NT treatment increased nutrient turnover through increased microbial biomass and enzyme activity. In addition, the same tendency in fungal communities of sugarcane leaf litter has been observed; NT increased fast-growing fungi (Miura et al. 2015). Consequently, our findings of higher concentration of microbial PLFAs, higher percentage of DNA metabolism-related sequences, and higher nutrient availability under the NT treatment suggest that the NT treatment enhanced microbial reproduction and increased nutrient turnover.

Microorganisms often use secondary metabolites (i.e., antibiotics (Fig. S2)) to compete with other microorganisms (Vining 1990), animal predators (Burkepile et al. 2006), and plants (Schippers et al. 1987). In addition, some microbial secondary metabolites (i.e., auxin (Fig. S2)) act as plant growth substances. Therefore, a higher percentage of secondary metabolism genes in soil under the NT treatment than under the CT treatment indicates that the NT practice activates biological interactions in soil.

The M treatment increased the percentage of sequences related to dormancy and sporulation, whereas it decreased the percentage of sequences related to amino acids and derivatives. The application of bagasse, which is a high C/N substrate, can limit microbial activity and metabolisms of amino acids because of N limitation. Blagodatsky and Richter (1998) indicated that dormant microbial biomass was higher in N-poor conditions than in N-rich conditions. In addition, the C and N from high C/N ratio residues are very slowly decomposed and only partially assimilated by the microbial biomass, while the C and N from low C/N ratio residues are rapidly assimilated by the microbial biomass and then become completely available for stabilization (Kirkby et al. 2013). Poor-N residues might then produce a reduced humification process per unit of added C because of the high proportion of recalcitrant compounds (Crawford et al. 1977; Kirkby et al. 2013). This can also explain why the C content of soils under the NTM treatment was not higher than soils under the NT treatment.

More sequences involved in sulfur metabolism were present in the NT- and M-treated soils. It is important to note that the NT- and M-treated soils also had a higher relative abundance of Proteobacteria, which contains most of the known species related to sulfur metabolism. Although sulfur is an essential element for the growth of plants, sulfur content in tropical soil is generally low, reflecting the poor SOM content (Sanchez et al. 2003). When sulfur content is increased alongside the increase in SOM content, microorganisms that are able to increase the bioavailability of sulfur can drive the cycling of this element.

Conclusion

This study indicated that the functional composition was less affected by agricultural treatments than the taxonomical composition of soil microbial communities. This result supports the hypothesis of the functional redundancy of soil microbial

communities. However, some metabolism-related sequences were affected by the NT and M treatments in association with changed soil properties, which supports our hypothesis. In addition, the shifts in the taxonomic and functional composition of soil microbial communities from the CT to NT treatment and from the CT to M treatment were clearer in the third-year samples than in the first-year samples. This indicates that the degree of the microbial shifts increases after prolonged management. Overall, our findings suggested that the microbial communities change their functionality in response to NT and M treatment, and these functional shifts have the potential to affect important soil processes that sustain crop productivity, such as C sequestration and major nutrient cycles. Based on our results, the NT management can be considered an important strategy for sustaining crop productivity, even in tropical soils. Nevertheless, further research will be needed to clarify how the taxonomical and functional profiles of soil microbial communities contribute to soil functioning under different agricultural managements.

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