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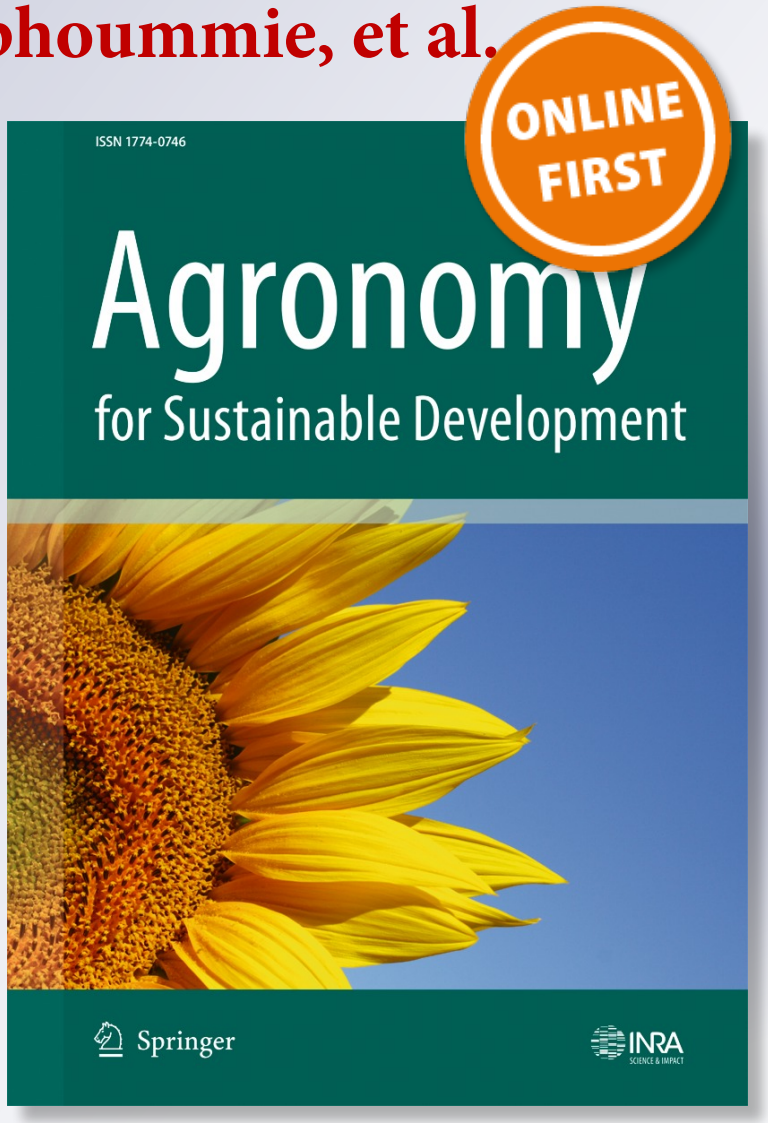

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RESEARCH ARTICLE

Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland

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Abstract It is widely assumed that agricultural practices have a major impact on soil living organisms. However, the impact of agricultural practices on soil microbes is poorly known, notably for species richness, evenness, and taxonomic composition. The taxonomic diversity and composition of soil indigenous microbial community can be assessed now using pyrosequencing, a high throughput sequencing technology applied directly to soil DNA. Here, we studied the effect of agriculture management on soil bacterial and fungal diversity in a tropical grassland ecosystem of northeastern Laos using 454 pyrosequencing of 16S and 18S rRNA genes. We studied soil microbial diversity of agricultural soils 3 years after conversion from native grasslands. We compared five systems: one tillage, two no-tillage rotational, one no-tillage improved pasture, and one natural grassland. Our results show first that compared to the natural grassland, tillage decreases fungal richness and diversity by −40 % and −19 %, respectively and increases bacterial richness and diversity by +46 % and +13 %, respectively. This finding evidences an early impact of agricultural management on soil microbial diversity. Such an impact fits with the ecological concept of "intermediate perturbation"—the hump-backed model—leading to classify agricultural practices according to the level of environmental stress they generate. We found also that land use modified soil microbial taxonomic composition. Compared to the natural pasture, tillage decreased notably the relative abundance of *Actinobacteria* (by −6 %), *Acidobacteria* (by −3 %) and *Delta-proteobacteria* (by −4 %) phyla, and by contrast increased the relative abundance of *Firmicutes* (by +6 %), *Gamma-proteobacteria* (by +11 %), and *Chytridiomycota* (+2 %) phyla. We conclude that soil microbial diversity can be modified and improved by selecting suitable agricultural practices. Moreover no-till systems represented intermediate situations between tillage and the natural pasture and appear therefore as a fair trade-off between the need for agriculture intensification and soil ecological integrity preservation.

Keywords Microbial diversity · Soil metagenomics · Pyrosequencing · Conservation agriculture · Tillage · Acid savannah

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1 Introduction

Among human activities, agricultural practices strongly affect soil microbial communities by changing the physical and chemical characteristics of the soil in which microorganisms live, thereby affecting their abundance, diversity, and activity (Kladivko 2001; Govaerts et al. 2007). Agricultural inputs (e.g., organic amendments, mineral fertilizers, and pesticides), crop rotation, and plant diversity affect soil microorganisms in different ways (Bunemann et al. 2006; Nicolardot et al. 2007; Pascault et al. 2013). However, in conventional agriculture, tillage generally has the greatest

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impact on biological properties since physical disturbance changes soil water content, temperature, aeration, and the degree of mixing of crop residues within the soil matrix (Kladivko 2001; Six et al. 2006). Tillage also reduces soil macroaggregate content (Tivet et al. 2013), which provides an important microhabitat for microbial populations (Ranjard and Richaume 2001). Based on the principle of minimal soil disturbance, no-till farming systems have been widely adopted in large-scale mechanized agriculture to prevent soil erosion and decrease production costs (Derpsch et al. 2010). Combined with soil cover (mulch) and diversified crop rotation, no-till systems are also being advocated over tillage for enhancing soil health and long-term crop productivity (Govaerts et al. 2007).

To date, the effects of agricultural practices and, more widely, of cropping intensity were mostly evaluated on soil microbial abundance and structure by using classical tools based on the cultivability of microorganisms as well as on their physiological and biochemical properties (Kladivko 2001; Kandeler 2007). However, these techniques were strongly limited in their sensibility and exhaustivity to give an accurate overview of quantitative and qualitative modifications of soil microbial communities (Maron et al. 2010). The recent development of culture-independent molecular tools, and especially of high throughput sequencing technology (pyrosequencing), allows obtaining thousands of sequences from a single soil DNA sample which may helped better assessing the huge diversity of soil microbial communities (Roesch et al. 2007; Terrat et al. 2011). Metagenomic analysis should facilitate the deciphering of taxonomic and functional assemblages of indigenous communities in natural environments, together with their roles in the biological functioning of ecosystems (Maron et al. 2010). To date, this approach has been poorly used to evaluate the impact of agricultural practices on soil microbial communities, hence limiting our interpretation of soil microbial taxonomic diversity changes, and their significance in terms of soil ecological status and potential functioning in agrosystems.

In a previous study, we showed that tillage systems and cover crops rapidly affected soil microbial abundance and genetic structure in a tropical grassland ecosystem of north-eastern Laos (Lienhard et al. 2013). However, the genotyping techniques used were unable to accurately characterize the full diversity of the telluric microbial communities. Consequently, they could not narrow the knowledge gap concerning the distribution and diversity (in terms of species richness and evenness) of indigenous microbial species in response to soil disturbance and cropping intensity.

In this study, our objective was to deepen the effect of agricultural management on soil microbial diversity by using a metagenomic approach. More precisely, our study aims at making an inventory of the diversity of both soil bacterial and fungal communities using new generation sequencing technology on soil DNA. For this, we compared soils coming from five contrasted land use management systems (one tillage-based and two no-till rotational cropping systems, one no-till improved pasture and the natural surrounding pasture), 3 years after the conversion of native grassland into agricultural land. Soil bacterial and fungal diversity were evaluated by 454 pyrosequencing of 16S and 18S rRNA genes, respectively. The analysis of microbial diversity changes, and notably the changes in particular taxonomic groups' distribution was used to evaluate agricultural systems effect on soil ecological status.

2 Material and methods

2.1 Experimental site and land use management

Experiments were conducted in Poa village (latitude, 19°33'N; longitude, 102°59'E) at 1,130 m AMSL. The climate is tropical and mountainous with a 6-month (April–September) wet and hot season and a 6-month dry season including 3 months of cold. The mean annual precipitation is 1,400 mm. The soils at the site are red clayey oxisols (USDA classification). We studied five land use management systems representing a decreasing gradient of cropping intensity and soil disturbance (Table 1): one tillage-based rotational system (CT), based on soil ploughing and repeated human interventions (e.g., sowing, manual weeding, and fertilizer application); two no-till rotational systems (NT1 and NT3) with limited soil disturbance (no-tillage) but also including frequent agricultural operations (e.g., rolling, spraying, sowing, and fertilizer application); one intensively grazed no-till and mono-specie improved pasture (ImpP); and a natural unfertilized and barely grazed grassland (PAS). The annual crop treatments (CT, NT1, and NT3) were selected from the split-split plot experimental design described in Lienhard et al. (2013). The pasture treatments (ImpP and PAS) were taken from surrounding fields (Fig. 1).

2.2 Soil sampling and chemical analysis

The soil was sampled at 0–10-cm depth, on the 29th of June 2010, 40 days after maize sowing. A composite sample was made of a pool of five subsamples taken in the diagonal section of the plot. For annual crop systems, soil was sampled on the maize row to avoid a possible "cover crop" effect under no-till systems. Soil chemical analyses were done by the INRA laboratory in Arras, France. Soil texture was measured by sieving methods (3 classes). Soil organic carbon (SOC) and total nitrogen (N) were quantified by dry combustion. Soil pH was measured in 1:5 soil/water slurry, and exchangeable bases (Ca, Mg, K, Na) were quantified using ammonium acetate reagent.

Table 1 Main land use management characteristics

Land use	Main characteristics
PAS	Native unfertilized pasture (>30 years) dominated by *Themeda triandra*, barely grazed (animal stocking rate <0.3 head/ha^{-1}) during the rainy season, and periodically burned during the dry season
ImpP	Improved pasture of ruzi grass direct seeded (no-till) in 2007 after the chemical control of native grasses; grazed (mean animal stocking rate of 4 heads/ha^{-1}) and fertilized[a] during the rainy season
CT	Conventional tillage: 3-year rotation of soybean (2008), rice (2009), and corn (2010) based on annual ploughing with discs, the burying of former crop residues and weeds, and mineral fertilization[a]
	No-till systems: similar to CT regarding crop rotation and fertilization level[a] but conducted under no-tillage, crop residue maintenance at soil surface, and the association of cover crops prior to and with main crops:
NT 1	Fm+Pp (2007), soybean+oat+buck (2008), rice+stylo (2009), corn+Pp (2010)
NT 3	Ruzi+Pp (2007), soybean+(oat+buck)+ruzi (2008), rice (+ stylo)+ruzi (2009), corn+ruzi (2010)

Main crops: rice cv. Sebota1, corn hybrid LVN10, soybean cv. Asca. Cover crops: Fm, finger millet (*Eleusine coracana* Gaern); Pp, pigeon pea (*cajanus cajan* cv. Thai); stylo, *stylosanthes guianensis* cv. CIAT 184; oat, *Avena sativa* L.; buck, buckwheat (*Fagopyrum esculentum* Moench); ruzi, ruzi grass (*Brachiaria ruziziensis* cv. ruzi)

[a] Annual fertilization of 60–80–60 kg ha^{-1} of N–P_2O_5–K_2O (32 kg N ha^{-1} for soybean). All agricultural treatments also received an initial application of 2 Mg ha^{-1} of locally produced lime (27 % of CaO)

2.3 Pyrosequencing of 16S and 18S rRNA gene sequences

Microbial DNA was extracted from 2 g (dry weight) of soil using a single procedure standardized by the GenoSol platform (INRA, Dijon, France, www.dijon.inra.fr/plateforme_genosol).

Microbial diversity was estimated by 454 pyrosequencing, a molecular technique allowing a rapid and massive production of targeted DNA sequences (Maron et al. 2010). A 16S rRNA gene fragment of the appropriate size (about 440 bp) and sequence variability for 454 pyrosequencing was amplified using the primers 338 F (5'-ACTCCTACGGGAGGCAGC AG-3') and 803R (5'-CTACCNGGGTATCTAAT-3') according to a procedure described by Terrat et al. (2011). Briefly, for each soil, 5 ng of DNA was used for a 25-μL PCR conducted under the following conditions: 94 °C for 2 min, 35cycles of 30 s at 94 °C, 52 °C for 30 s and 72 °C for 1 min, followed by 7 min at 72 °C. PCR products were purified using a MinElute gel extraction kit (Qiagen, Courtaboeuf, France) and quantified using the PicoGreen staining kit (Molecular Probes, Paris, France). Similarly, a 18S

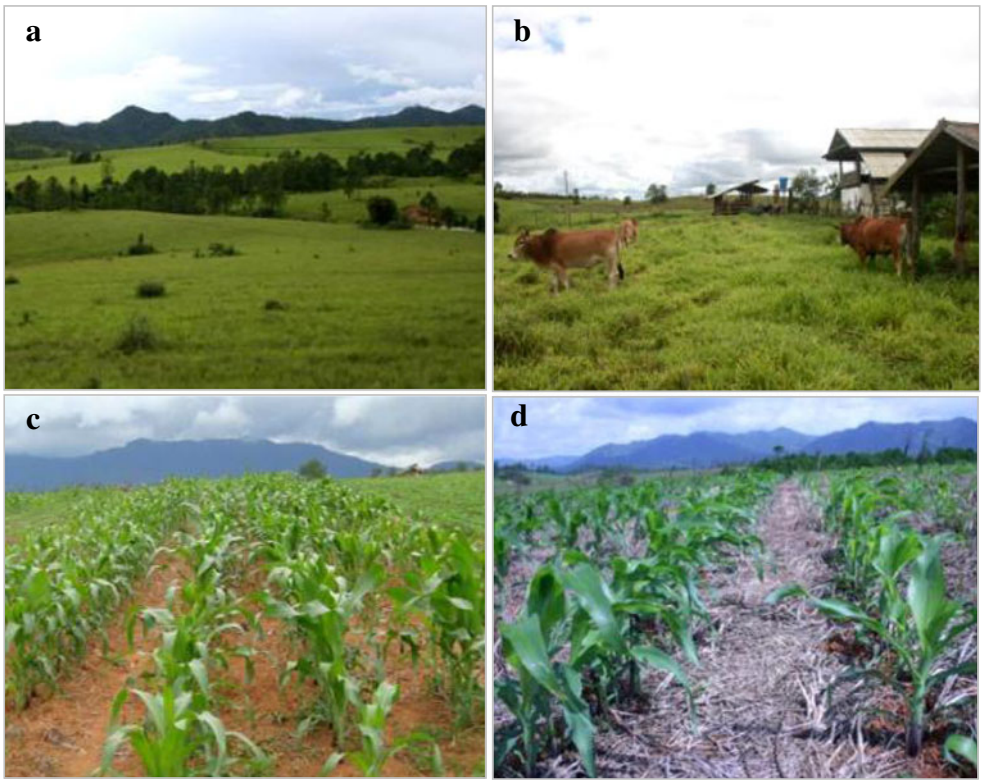

Fig. 1 Land use management sampled in Poa experimental site. **a** Natural unfertilized pasture (*PAS*), **b** improved pasture of *Brachiaria ruziziensis* (*ImpP*), **c** maize crop under tillage (*CT*), and **d** maize crop under no-till system (*NT1* and *NT3*)

rRNA gene fragment of about 350 bp was amplified using the primers FR1 (5'-ANCCATTCAATCGGTANT-3') and FF390 (5'-CGATAACGAACGAGACCT-3') under the following PCR conditions: 94 °C for 3 min, 35cycles of 1 min at 94 °C, 52 °C for 1 min and 72 °C for 1 min, followed by 5 min at 72 °C. A second PCR of nine cycles conducted under similar conditions was then realized with purified PCR products and 10 bp multiplex identifiers added to the primers at 5' position to specifically identify each sample and avoid PCR biases. PCR products were finally purified and quantified as previously described. Pyrosequencing was then carried out on a GS Junior (Roche 454 Sequencing System).

2.4 Bioinformatic analysis of 16S and 18S rRNA gene sequences

Bioinformatic treatment was done using the GnSPipe of the GenoSol platform (INRA, Dijon, France) described by Terrat et al. (2011). Firstly, all reads were sorted according to the chosen identifiers' sequences. Then, in order to efficiently compare the datasets and avoid biased community comparisons, raw datasets which had a large number of reads were reduced by random selection close to the lowest datasets (8,000 and 2,500 reads for 16S and 18SrRNA gene sequences, respectively). Raw reads were filtered and deleted: (a) if the exact primer was not found at the beginning of the sequence, (b) if the sequences contained any ambiguity (Ns), (c) if its length was below 350 and 250 bases for 16S and 18S reads, respectively. A PERL program was then applied to obtain strict dereplication (i.e., clustering of strictly identical sequences). The dereplicated reads were then aligned using infernal alignments and clustered into molecular operational taxonomic units (MOTU) using a PERL program that clusters rare reads to abundant ones and do not count differences in homopolymer lengths. Another homemade filtering step was then applied to eliminate potential sources of errors (e.g., PCR chimeras, sequencing errors, MOTU overestimation). All single-singletons (reads detected only once and not clustered) were then checked based on the quality of their taxonomic assignments to avoid artifacts. High-quality reads were then used for taxonomy-based analysis using: (a) the Naïve Bayesian rRNA classifier of the RDP project for bacterial sequences, (b) the Basic Local Alignment Search Tool performed on a cleaned version of the Silva database (version r111 using the EMBL taxonomy) for fungal sequences. Diversity indexes were finally determined using the detected taxonomic groups at the genus level. We used the maximum number of MOTU, the Shannon (H'), and Evenness (J) indexes as indicators of soil microbial richness, diversity and structure, respectively. The raw data sets are available on the EBI database system (and Short Read Archive) under project accession number ERP002181.

2.5 Statistics

Principal component analysis (PCA), were performed using the ADE-4 package (Thioulouse et al. 1997) under R software and provided an ordination of data in factorial maps based on the scores of the first two principal components.

3 Results and discussion

3.1 Effect of agricultural management on soil chemical characteristics

Despite the limited time of cultivation at evaluation (3 years), we observed a significant early effect of agricultural systems on top soil chemical characteristics (Table 2). We recorded a rapid decrease in SOC and total nitrogen (N) under conventional tillage, with a mean loss of 25 % of SOC and total N under conventional tillage as compared to no-tilled systems, which may be related to macro-aggregate disruption, enhanced soil aeration, and the mixing of residues into the soil (Six et al. 2006). We also observed an increase in soil pH and exchangeable base content under all cultivated systems as compared to the natural pasture (Table 2) due to lime and thermophosphate supply (Table 1). However, soil exchangeable base content was much higher under no-till cultivated systems (NT1, NT3, and ImpP) than under conventional tillage (+75 % in average; Table 2), suggesting important nutrient losses by lixiviation under tillage systems. Altogether, our results are consistent with other studies comparing till vs no-till system effect on soil chemical properties at soil surface (Kladivko 2001; Govaerts et al. 2007).

3.2 Response of soil microbial diversity to land conversion to agriculture

Regardless of agricultural systems, the examination of the clustered DNA sequences revealed low microbial richness in Lao soils, with less than 550 MOTU, detected for both bacterial and fungal communities (Table 2), whereas more than 1,000 MOTU were described in other soil ecosystems for both bacterial (Acosta-Martinez et al. 2008; Terrat et al. 2012; Tripathi et al. 2012) and fungal (Buee et al. 2009) communities. This low richness could be related to the soil characteristics, since microbial diversity is strongly influenced by soil pH (Lauber et al. 2009), Al toxicity, and nutrients availability (Tripathi et al. 2012).

After 3 years of native grassland conversion to agriculture, we observed an early but significant effect of land use management on bacterial and fungal diversity (Table 2). Bacterial diversity was favoured by increased soil disturbance and cropping intensity, with values of the Shannon index (H') and richness (MOTU) decreasing along the gradient conventional

Table 2 Top soil (0–10 cm) chemical characteristics, microbial molecular abundance, and diversity according to land use management

Land use	Clay (%)	pH H_2O (1:5)	Soil organic content (g kg^{-1})	N tot (g kg^{-1})	C/N	Σ base[a] (cmol kg^{-1})	Microbial diversity					
							Bacterial			Fungal		
							MOTU	H'	J	MOTU	H'	J
PAS	57	4.9	38.6	2.6	14.8	2.1	361	3.9	0.67	392	4.8	0.80
ImpP	59	5.1	36.8	2.5	14.8	4.2	431	4.0	0.67	335	4.3	0.73
CT	55	5.0	28.9	1.9	15.0	2.5	528	4.4	0.70	236	3.9	0.72
NT1[b]	60	5.3	39.3	2.7	14.4	4.6	507	4.3	0.70	467	4.9	0.80
NT3[b]	63	5.2	45.1	3.1	14.5	4.3	477	4.4	0.72	341	4.5	0.78

PAS natural pasture, *ImpP* improved pasture, *CT* conventional tillage, *N tot* total nitrogen, *C/N* carbon to nitrogen ratio, *MOTU* molecular operational taxonomic units corresponding to richness determined at the genus level, *H'* Shannon index, *J* Evenness index

[a] Sum of exchangeable bases (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+})

[b] No-till system

tillage>no-till systems>improved pasture>natural pasture (Table 2). As compared to the natural pasture, conventional tillage increased H' value by +13 % (from 3.9 to 4.4) and bacterial richness by +46 % (from 361 to 528 MOTU, Table 2). Interestingly, this gradient was the opposite of the results observed for bacterial density (Lienhard et al. 2013).

According to the "hump-backed" model that links the biodiversity of a community to the intensity of its exposure to environmental stress (Giller et al. 1998; Bressan et al. 2008), our results suggest that the tillage events may at that stage represent a moderate perturbation for bacteria leading to a decrease in the competitive niche exclusion and selection mechanisms occurring between populations, and consequently to an increase in bacterial richness. In other respects, plant diversity (with no-till systems>improved pasture; Table 2) and mineral fertilization (with no-till systems and improved pasture>natural pasture) may also have contributed to increased bacterial diversity under no-till agricultural systems as compared to the native grassland due to their positive effect on soil pH and nutrient availability (Table 2) which have been shown to favour bacterial diversity (Lauber et al. 2009; Tripathi et al. 2012).

Contrary to bacterial diversity, fungal diversity was negatively affected by tillage, with lower values of richness (MOTU) and Shannon index (H') observed under conventional tillage (236 MOTU and H' of 3.9) than under no-tilled systems (MOTU ranging from 335 to 467, and H' from 4.3 to 4.9; Table 2), which might be related to the negative effect of tilling tools on fungal hyphal growth (Six et al. 2006). We found notably twofold higher fungal richness and 25 % higher H' value under no-till system 1 than under conventional tillage (Table 2). Plant diversity also appeared important in maintaining soil fungal diversity, with 40 % higher fungal richness and 14 % higher H' value observed under no till system 1 as compared to the improved pasture (Table 2), this finding being in agreement with Nishizawa et al. (2010) who observed similar correlations between plant and fungal diversity.

Finally, although the land use management did not modify 2 years after grassland conversion into agricultural land the fungal to bacterial density ratio (Lienhard et al. 2013), it deeply impacted the fungal to bacterial diversity ratio, with the highest bacterial diversity and the lowest fungal diversity observed under conventional tillage (Table 2). Interestingly, this suggests that diversity measurements are more sensitive than density measurements to evidence early impacts of land use management on soil microbial properties.

We used the "hump-backed" relationships between biodiversity and the level of environmental stress (Giller et al. 1998; Bressan et al. 2008) to classify the agricultural systems according to their impact on soil microbial diversity (Fig. 2). Conventional tillage represented a moderate perturbation for bacteria and a high perturbation for fungi, whereas no-till and improved pasture systems represented a lower perturbation for both bacterial and fungal populations. No-till cropping systems represented intermediate situations between tillage and monospecies improved pasture systems in terms of environmental perturbation. Altogether, our results suggest the promotion of no-till systems as a fair trade-off between the need for agriculture intensification and soil biological integrity preservation.

3.3 Effect of agricultural management on soil bacterial and fungal taxonomic composition

Although the number of bacterial and fungal phyla were not significantly different between the different agricultural management (data not shown), we observed an early and strong effect on their relative abundance (Fig. 3a, b). This observation was confirmed by PCA based on taxonomic composition, with a clear discrimination of land use on the factorial maps (Fig. 3c, d). Interestingly, we observed similar gradients between bacterial diveristy (Table 2) and

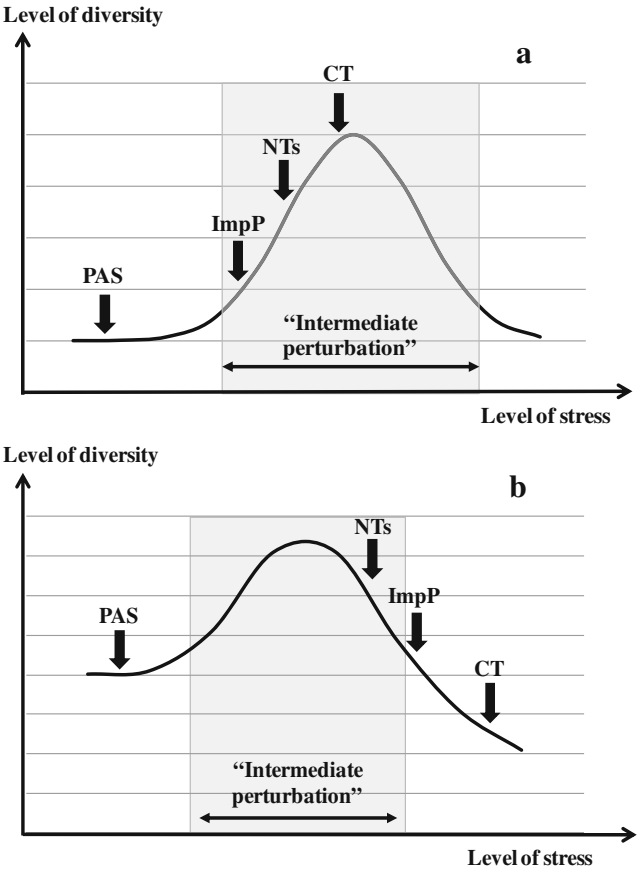

Fig. 2 Schematic representation based on the "hump-backed" model of the level of **a** bacterial and **b** fungal diversity according to the level of environmental stress induced by the different agricultural systems. Natural pasture (*PAS*), improved pasture (*ImpP*), conventional tillage (*CT*), no-till systems (*NTs*)

composition (Fig. 3c), with conventional tillage being strongly discriminated from pasture on the first PCA axis, and no-till and improved pasture representing intermediate situations. This highlights the importance of soil disturbance and cropping intensity as major drivers of soil bacterial diversity in our agricultural systems.

Regardless of land use management, the *Proteobacteria* was the dominant bacterial phyla and represented 35 to 45 % of all bacterial DNA sequences (Fig. 3a). Compared to the natural pasture, we observed a decrease of this phyla (by −6 %) under conventional tillage, which may be explained by a reduced soil organic carbon content (Table 2) since the *Proteobacteria* have been described as fast growing copiotrophs stimulated in C-rich environments (Bernard et al. 2007; Cleveland et al. 2007; Fierer et al. 2007; Jenkins et al. 2010). Agricultural management also affected the class distribution within this phyla, with a higher relative abundance of *Gamma-proteobacteria* (+11 %) and a lower relative abundance of *Alpha-* (−7 %), *Beta-* (−6 %), *and Delta-proteobacteria* (−4 %) observed under conventional tillage as compared to the natural pasture (Fig. 3c). In addition, improved pasture was discriminated from the other no-till systems on the second PCA axis because of a higher relative abundance of *Beta-proteobacteria* (Fig. 1c), and notably of *Massilia* genus (25 % of all sequences), which has been described as a root colonizing bacteria stimulated by root exudates and readily degradable carbon compounds (Ofek et al. 2012). This is consistent with Wenzl et al. (2001) who found ruzi grass roots to exudate a high amount of organic acids (e.g., malate, citrate, and oxalate) as a mechanism of Al resistance.

The *Actinobacteria* was the second most abundant phyla with 30 to 40 % of all the bacterial sequences (Fig. 1a). *Actinobacteria* has been described as mainly K strategists (Bernard et al. 2007; Pascault et al. 2013), and well represented in non-disturbed grass systems (Acosta-Martinez et al. 2008; Yu et al. 2011). The high proportion of detected *Actinobacteria* in each system may consequently result from the pasture history of the site and the limited duration of cultivation (3 years) since conversion. However, as for the *Proteobacteria*, land use management shaped *Actinobacteria* distribution, with a decrease in relative abundance observed under conventional tillage as compared to Pasture (by −6 %; Fig. 3a, c). This may be explained by the particular morphology of those organisms, which are forming structures harbouring similarities with fungal hyphae (Stackebrandt et al. 1997) that make them more sensitive than other bacterial groups to physical soil disturbance.

Other bacterial phyla were also affected by land use management with a decrease in the relative abundance of *Acidobacteria* (by −3 %) observed under conventional tillage as compared to pasture, and by contrast, an increase in the relative abundance of the *Firmicutes* (by +6 %; Fig. 3a) that further distinguish conventional tillage and pasture on the first axis of the PCA (Fig. 3c). Bacteria belonging to the *Acidobacteria* phyla have been described as mainly oligotrophs (K-strategists), which utilize complex carbon substrates that are more likely to be present in the native SOM (Bernard et al. 2007; Fierer et al. 2007). In addition, *Acidobacteria* abundance has been shown to increase with soil age (Nemergut et al. 2007), which may explain their higher abundance in the undisturbed pasture treatment. By contrast, the *Firmicutes* have been described as mainly copiotrophs (Bernard et al. 2007; Cleveland et al. 2007; Fierer et al. 2007; Jenkins et al. 2010), which are, however, able to survive in adverse environmental conditions due to their ability to produce endospores (Mandic-Mulec and Prosser 2011). This may explain that their stimulation under conventional tillage represents the most disturbed and most carbon-depleted environment.

Regarding fungal taxonomic diversity, the dominant phyla were the *Ascomycota* (55–65 % of all fungal sequences; Fig. 3b), and the *Basiodiomycota* (20–35 % of all fungal sequences). These two phyla mainly belong to the saprotrophic soil fungi (de Boer et al. 2005) and are classically dominant in

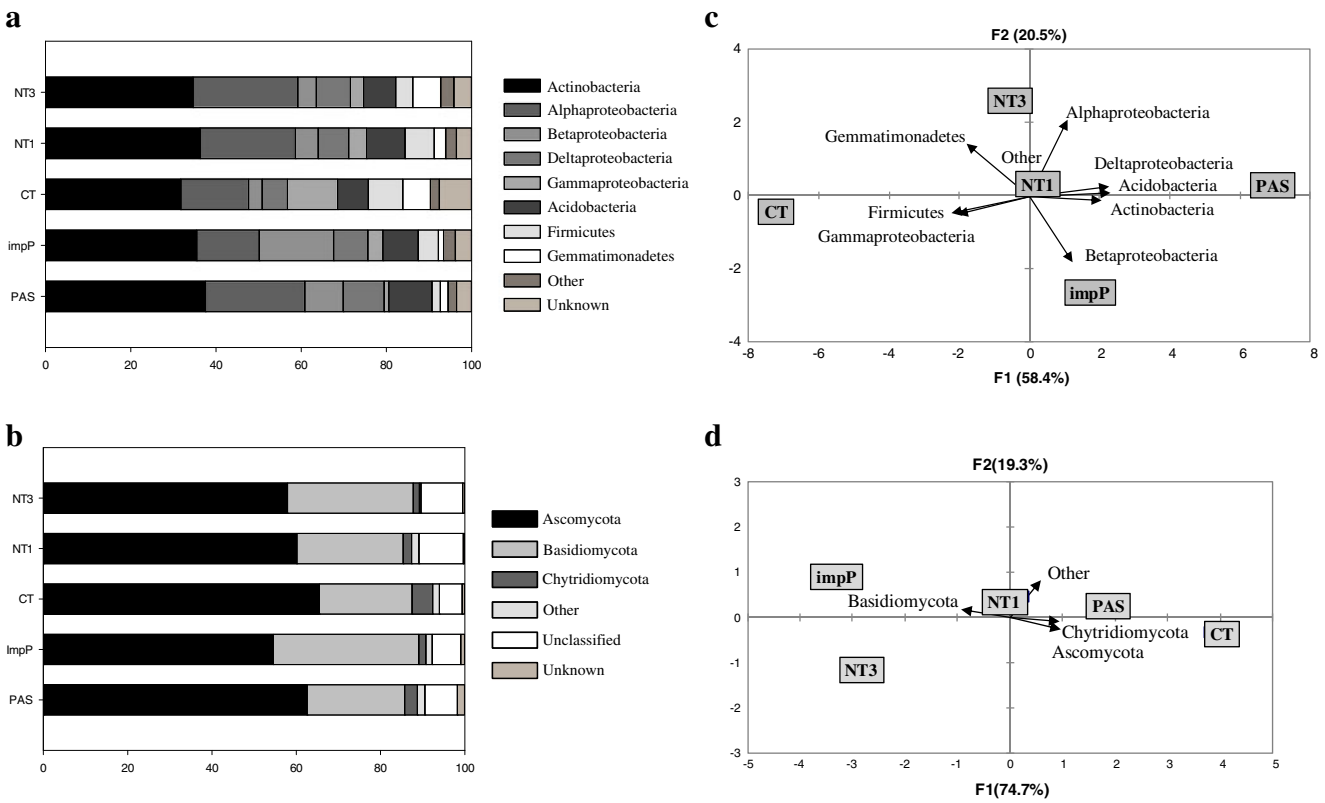

Fig. 3 Bar plot representation of **a** soil bacterial and **b** soil fungal phyla relative abundance according to land use management, and factorial maps of the PCA performed on **c** soil bacterial and **d** soil fungal phyla relative abundance. Natural pasture (*PAS*), improved pasture (*impP*), conventional tillage (*CT*), no-till systems (*NT1* and *NT3*)

soils (Buee et al. 2009; Nishizawa et al. 2010; Yu et al. 2011). However, land use management affected phyla relative abundance distribution, with a higher relative abundance of *Basidiomycota* observed under ruzi grass-dominated systems as compared to the conventional tillage (+12 % and +8 % for improved pasture and no-till system 3, respectively), and by contrast a lower relative abundance of *Ascomycota* (−11 % and −7 %, respectively) and *Chytridiomycota* (−3 % and −3.5 %, respectively) under improved pasture and no-till system 3 as compared to conventional tillage (Fig. 3d). Our results are consistent with de Boer et al.'s (2005) description of *Basidiomycotina* fungi's predominant role in lignin degradation, with a higher amount of stubble restitution under improved pasture and no-till system 3 than under conventional tillage (Lienhard et al. 2013). In addition, soil *Chytridiomycota* have been shown to be able to recover from drying and high temperature events (Gleason et al. 2004), which may more likely occur under bare and tilled soils (Six et al. 2006).

Altogether, the analysis of soil microbial phyla distribution gives us an interesting overview of the ecological status at evaluation through the ecological attributes of the microbial groups that were stimulated. It is worth noting that all the subtaxa of these broad phyla may clearly not conform to these general ecophysiological characteristics (oligotrophic vs copiotrophic attributes). However, our results suggest that general ecological attributes may be ascribed at the phyla level, in agreement with other authors (Cleveland et al. 2007; Fierer et al. 2007; Jenkins et al. 2010).

4 Conclusion

In an acid tropical grassland environment, we observed an early and significant effect of agricultural management on soil microbial properties, with tillage decreasing fungal richness and diversity, but increasing bacterial richness and diversity. We found also that land use modified soil microbial taxonomic composition. Compared to the natural pasture, tillage decreased notably the relative abundance of *Actinobacteria*, *Acidobacteria*, and *Delta-proteobacteria* phyla, and by contrast increased the relative abundance of *Firmicutes*, *Gamma-proteobacteria*, and *Chytridiomycota* phyla. Consequently, our results highlight that no-till cropping systems represented intermediate situations between tillage and the natural pasture, and therefore suggest the promotion of no-till systems as a fair trade-off between the need for agriculture intensification and soil ecological integrity preservation.

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