Functional and molecular diversity of rice straw decomposing bacteria and fungi

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Summary

The Mekong Delta is one of two rice baskets in Vietnam. Due to the convenient environmental conditions for rice cropping, people in this region have cultivated two or three rice crops a year. The intensive rice cultivation may have a negative impact on the soil environment and soil microbial diversity. To start investigations on how to reduce negative impacts on the soil environment and maintain the soil fertility in a long term perspective, this study was made. Rice stubble was collected from two different rice fields, Hoa An (Acid sulphate soil) and Vinh Nguon in the Mekong Delta. The functional traits and molecular diversity of cultivable bacteria and fungi as well as their effects on the next rice crop in terms of germination and radicle length were investigated. The effect of increasing number of species and functional groups was also studied to understand the relationship between microbial diversity and function. Microcosms containing sterile rice straw inoculated with taxonomically and functionally different microorganisms were used for this purpose.

A total of 259 bacterial strains and 45 fungal isolates were identified, with various functional characteristics. Cellulolytic and chitinolytic activity was abundant among bacteria but fluorescent isolates were absent. The rice stubble from the Hoa An site exhibited a higher functional diversity of bacteria compared to that at the Vinh Nguon site mainly because of high abundance of chitinolytic and cellulolytic bacteria while the fungal isolates in stubble from the Vinh Nguon site showed a higher functional diversity than that of the Hoa An site. Bacteria that were either deleterious, neutral or beneficial to rice seed emergence and radicle growth were found. The proportion of deleterious bacteria was almost 3 times higher than that of beneficial ones. In contrast, the majority of the isolated fungi seemed to be beneficial at the inoculum concentration tested. Molecular diversity of 259 bacterial and 45 fungal isolates was assessed by sequencing the 16s rDNA and the ITS rDNA respectively and showed that bacterial strains could be sorted into 17 families and the fungal isolates belonged to 9 families. Taxonomic groups on the family level generally had a high bootstrap support in a Neighbour-joining analysis. The bacterial communities differed significantly among the two sites with the highest molecular diversity in the acid soil. *Bacillaceace, Burkholderiaceae, Enterobacteraceae* and *Pseudomonadaceae* were the most common families. Functional traits were found in phylogenetically diverse families. Cellulolytic activity was found in the *Bacilliaceae, Enterobacteriaceae, Flexibacteraceae, Microbacteriaceae* and the *Paenibacillaceae*. The chitionolytic activity was most prevalent within the members of *Flexibacteraceae* but present in *Burkholderiaceae, Enterobacteriaceae, Oxalobacteriaceae, Staphylococcaeae* and *Xanthomonadaceae*. Fungal diversity did not differ among the two sites but species composition was different. Potential rice pathogens within the *Nectriaceae* and *Trichocomaceae* families were also isolated from the straw.

The results from the microcosm study revealed that the straw inoculated with either single cultures or various mixtures exhibited a dry weight loss up to 32% depending on the

treatment. There was a positive relationship between the number of species inoculated and the weight loss. There was also a positive correlation between the number of functional groups and weight loss as well as between functional dissimilarity and weight loss**.** Multiple regression analysis showed that in addition to the number of species and functional groups, 5 fungal species and one bacterial species contributed significantly to the decomposition of rice straw. Highest weight loss was achieved in microcosms inoculated with fungi. The decomposition by bacterial inocula was lower than for fungi indicating that the presence of bacteria had an inhibiting effect on the total decomposition during the experimental period. My results using mixtures of fungi and bacteria agree well with previous observations reporting a positive response of the number of species on degradation rates for either fungal or bacterial biodiversity. Functional redundancy was not a general phenomenon in our experiment since many single cultures and species mixtures showed low levels of degradation. The results are consistent with the well accepted view that fungi are the most important contributors to the degradation of recalcitrant plant material in terrestrial environments. In this experiment, interactions between fungi and bacteria seemed to be mostly neutral but with examples of both positive and negative interactions. It is likely that both facilitative interaction and species effects contribute to the positive relationship between species/ functional biodiversity and rice straw decomposition.

Introduction

The Mekong Delta in Vietnam has 4 million hectares of very intensively managed agriculture based on rice production that is either irrigated or rain fed. Large quantities of rice straw are thus produced. Straw decomposition depends on the colonization and growth of microorganisms able to degrade cellulose and lignin. Decomposed rice straw is an important substrate for soil microorganisms as well as for the recovery of soil fertility owing to the recycling of carbon, nitrogen and other nutrients back to the soil. Substituting rice straw burning with efficient decomposition of straw could reduce soil damage from intensive cropping.

Crop residues added to and decaying in soil have various compositions and comprise of complex polymers such as cellulose and lignin. The general microbial processes involved in decomposition of residues may occur under both aerobic and anaerobic conditions. For most soils, the aerobic pathway is of greater importance than the anaerobic one. More complex and resistant substrates, such as carbohydrate polymers, may initially attract a group of microbes that breaks down the polymer into simpler components. This is followed by the activity of other groups of microorganisms that can use the simpler components. The final step is the assimilation of decay products to be utilized by diverse microbes that oxidize the compounds to obtain energy and carbon for the production of new tissue. (Wolf and Wagner, 2005)

Soil fungi comprise an extremely diverse group of microorganisms. About 700 species representing 170 genera have been identified in soils, (Frosyth and Myyata, 1996). The number of bacterial species is in the order of hundreds to thousands in one gram of soil; the total species number is estimated at 2 to 3 million (Torsvik *et al.* 1994, Dejonghe *et al.* 2001). Species diversity of soil fungi is probably only slightly less than that of bacteria (Bridge & Spooner 2001, Hawksworth 2001).

The role of bacteria and fungi in cellulose degradation

Cellulose is the main polysaccharide in terrestrial ecosystems. Rice straw has a cellulose content of 37 - 49% (Watanabe *et al.*, 1993). It represents a huge source of energy for microorganisms. In nature, most cellulose is degraded aerobically and the final product is CO_2. Cellulose is insoluble in water and therefore requires enzymatic degradation. The ability of bacterial and fungal communities to degrade cellulose aerobically is widespread among some soil microbial groups. Examples of cellulose degrading bacteria are found in both filamentous (e.g. *Streptomyces, Micromonospora*) and non-filamentous (e.g. *Bacillus, Cellulomonas, Cytophaga*) genera (Lynd *et al.*, 2002). Cellulose degradation is a common trait among fungi within both Ascomycota and Basidiomycota (Rayner and Boddy, 1988; Cooke and Rayner 1984; Lynd *et at.*, 2002). Aerobic cellulolytic fungi and bacteria produce freely diffusible extracellular cellulase enzyme systems consisting of endoglucanases,

exoglucanases and β- glucosidases that act synergistically in the conversion of cellulose to glucose (Lynd *et al.*, 2002; Mansfield and Meder, 2003). Although components of the cellulolytic system of soil bacteria and fungi can be distantly related, their function is quite similar (de Boer *et al*., 2005). With addition of cellulose to an agricultural soil, an initial phase featuring predominantly bacterial cellulose decomposition has been recognized, followed by a stage dominated by fungal cellulose decomposition (Minerdi *et al.*, 2001).

The role of bacteria and fungi in lignin degradation

Lignin, the most recalcitrant plant cell wall component, was present already in the oldest land plants. Rice straw is poorly degraded by many microorganisms although its lignin concentration is about 52g/kg which is lower than that in wheat straw (85-140 g/kg) and barley straw (110g/kg), respectively (data from Soest, 2006). The enzymes degrading lignin are oxidative, non-specific and act via non-protein mediators in contrast to hydrolytic cellulases and hemicellulases (Aro *et al.*, 2004). Several genera of Basidiomycota that are collectively named white-rot fungi can decompose lignin (Rayner *et al.*, 1988; Worrall *et al.*, 1997; Leonowicz *et al.*, 1999; Tuomelä *et al.*, 2000). They release enzymes such as laccases and peroxidases, and free radicals to break down lignin. This process occurs under strictly aerobic conditions. Bacteria also have an important role in lignin degradation although this role is considered negligible in terrestrial environments compared to that of white-rot fungi. (Tuomelä *et al.*, 2000 and Ce´spedes *et al.*,1997). However, the growth of both filamentous and non-filamentous bacteria on lignin-like compounds has been observed (Ce´spedes *et al.*, 1997; Falco´n *et al.*, 1995;] Vicun˜a, *et al.*, 1993; Peng *et al.*, 2002). Several actinomycetous species have also been shown to solubilise lignin, in particular lignin in grass species (Tuomelä *et al.*, 2000; Trigo and Ball 1994)

In addition to plant residues, the cells of dead microflora are, together with microfauna, are substrates for living microorganisms. Fungal cell walls composed of cellulose, chitin, and/or chitosan can be degraded. Chitin is biosynthesized in soils as an important cell wall component of common soil fungi, e.g., *Aspergillus* and *Penicillium* with from 3% to 25% of fungal biomass consisting of chitin on a dry-weight basis. The degradation rate of chitin in soil appears to be similar to that of cellulose**.** In aerobic soils, the dominant chitin degraders appear to be actinomycetes of the genera *Streptomyces* and *Norcardia.* Fungal genera, such as *Trichoderma* and *Verticillium* or bacteria, such as *Bacillus* and *Pseudomonas* have also been shown to degrade chitin, but appear to be less important than the *actinomycetes* (Wolf and Wagner, 2005). De Boer et al (1998) indicated that fungi used for in *invitro* tests of antagonism were more often affected by chitinolytic bacteria than by non-chitinolytic ones. The production and secretion of chitinase by non-pathogenic microorganisms may be

important in the biological control of plant pathogenic fungi (Viswanathan and Samiyappan, 2000).

Molecular techniques to study soil microbial communities

Traditional approaches to studies of microbial diversity have relied on laboratory cultivation of microorganisms from natural environments and identification by classical techniques, including analysis of morphology, physiological characteristics and biochemical properties (Prosser, 2002). It has been shown that only few microorganisms are cultivable (1-5% for bacteria) compared to the total diversity of taxa found in natural habitats. Phylogenetic types of environmental clones reveal about 5-52% of the microorganisms naturally present (Hengstmann *et al.*, 1999; Liesack and Stackebrandt 1992; Ward *et al.*, 1995; Amann *et al.*, 1995). Direct nucleic acid extraction approaches have in recent years been widely applied in soil microbial ecology. These techniques have provided a significant advance in our understanding of genetic diversity in soil microbial communities.

Analysis of 16S rRNA genes is now widely used for analysis of bacterial populations, and analyses of 18S rRNA genes and internal transcribed spacer (ITS) regions are increasingly being used to analyze fungal populations (James 2002). Some investigations on microbial communities in rice paddy soils have been performed, using both cultivation-independent molecular and cultivation-dependent techniques to identify bacterial communities (GroMkopf *et al.*, 1998; Chin *et al.*, 1999; Janssen *et al.*,1997; Henckel *et al.*, 1999). Kimura *et al.*, (2001) indicated Gram-positive bacteria as major decomposers of rice straw that was incorporated into paddy soil microcosms under submerged conditions. In contrast, gram negative bacteria and fungi are responsible for the decomposition of leaf sheaths and blades under oxic conditions in upland soils (Asumi *et al.*, 2003). RNA stable isotope probing revealed that the bacteria actively assimilating C from pulse-labeled rice plant were *Azospirillum* spp. (Alphaproteobacteria) and members of the family *Burkholderiaceae* (Betaproteobacteria). These degraders were present in high abundance in the rice root environment (Yahai *et al.*, 2006)

Effect of microbial functional groups on diversity

Diversity is the range of significantly different kinds of organisms and their relative abundance in natural habitats. Theoretically, diversity can be regarded as the amount and distribution of genetic information in a natural community. A representative estimate of microbial diversity is a prerequisite for understanding the functional activity of microorganisms in such systems (Garland and Mills, 1994; Zak *et al.*, 1994). The effects of different species on a particular process often differ in strength. These differences may lead to inter-specific interactions that result in species mixtures performing better (facilitative

interactions) or worse (inhibitory interactions) than would be expected on the basis of the mere additive effects of several single species. The nature of these interactions (inhibitory, neutral, or facilitative) might be related to the degree in which these species differ in their impact on soil processes (Heemsbergen *et al.*, 2004). Although the aforementioned studies clearly show that the competitive interactions between fungi and bacteria can be important during decomposition of more or less recalcitrant organic matter, the interactions are not necessarily always competitive. If fungal growth is constrained by other factors than the availability of soluble carbohydrates, bacterial consumption of these compounds may not be negative (Huston 1997; Loreau 1998; Wardle 1999; Huston *et al.* 2000; Kinzig *et al.* 2001).

There is much discussion about whether the observed positive relationship between diversity and function which was found in some studies was due to the importance of species diversity as such, or whether the specific properties of individual species is of overriding importance (e.g. Aarssen 1997; Huston 1997; Wardle 1999; 2002). For example, in studies on the effect of bacterial co-inoculation into spruce wood blocks with white rot fungi (*Heterobasidion annosum*, *Resinicium bicolor*, or *Hypholoma fasciculare*), the degradation of the wood consistently tended to be higher in co-inoculated traits than in traits inoculated with fungus alone, even though wood decay could not be ascribed to the bacteria by themselves (Murray and Woodward, 2003). Bacteria may positively affect fungal activity either by producing protease, cellulase and pectinase enzymes, the activity of which increases the accessibility of substrate to the fungus or stimulates the decomposition of inhibitory fungal decay products. The activity of organotrophic, nitrogen-fixing bacteria may increase the nitrogen levels available for fungal growth (Greaves 1971; Hendrickson 1991). Wohl *et al.* (2004) found that in a constant environment, greater species richness of cellulolytic bacteria supported a greater number of individuals and subsequently higher rates of total cellulose degradation.

The aims of study

The aim of this study was to link microbial diversity to the ecosystem function of rice straw decomposition. Microbial diversity is less studied in tropical soils compared to temperate and boreal soils. Since rice is predominantly grown in tropical or subtropical areas of the world, the microbial flora of rice fields deserves more scientific attention.

In this study, the aerobic microbial community associated with degradation of rice straw was investigated in Vietnamese paddy fields. The cultivable fungi and bacteria as main components of the community were

- Compared on rice straw from two different sites (Hoa An and Vinh Nguon) in the Mekong Delta, by using molecular taxonomy and characterisation of functional traits
- Investigated for relationships between functional diversity and microbial decomposition activity. Single isolates as well as combinations of different functional

groups of bacteria and fungi were investigated with respect to their effects on decomposition of rice straw under aerobic conditions.

Materials and methods

Isolation of rice stubble associated microorganisms

Rootless stubble of rice plants was used for isolation of microorganisms. Rice stubble was obtained one week after the harvesting of rice seeds from two fields (at the Vinh Nguon and Hoa An sites). The soils were chemically characterized in the laboratory of the department of Soil Science and Land Management, Cantho University, Vietnam (Table 1). Stubble was sampled from a rectangular grid 8 x 12 m at 1 m intervals. One corner of the grid was randomly chosen. In total 48 samples were collected for isolation. The stubble was cut into pieces of approximately 2 cm length. It was thoroughly rinsed with distilled water to remove all debris before surface-sterilization in 7.5% sodium hypochlorite for 1 min and repeated thorough rinsing with sterile distilled water, SDW. The stubble was cut further into small pieces and aseptically incubated on sterile Hagen agar (HA, NH_4NO_3 0.5g/l, KH_2PO_4 0.5g/l, $MgCl_2$. $7H_2O$ 0.5g/l, malt extract 5g/l and agar 15g/l (both from Merck) in distilled water) amended with sodium carboxymethylcellulose (10g/l) for isolation of cellulolytic fungi. Similarly, for isolation of cellulolytic bacteria, surface-sterilized stubble was crushed in sterile 0.01M $MgSO_4$ solution and the suspension thus obtained was serially diluted and spread on sterile cellulose amended TSBA (tryptic soybroth 10g/l, and 15 g agar per liter distilled water, both from Merck). The inoculated plates were incubated at 25°C and bacterial and fungal colonies as they appeared were further purified on TSBA and HA, respectively. Cultures were maintained on TSB for bacteria and HA for fungi at 4°C.

Functional characterization of rice stubble colonizing bacteria and fungi

Isolated bacteria and fungi were tested for four functional traits: cellulolytic, chitinolytic, fluorescence and effect on rice seed germination.

Enzymatic activity

To examine if all colonies appearing on cellulose amended media were cellulolytic, fresh pure cultures were treated with 0.1% congo red stain followed by flooding in 1M NaCl according to a procedure described by Alström (2000). Appearance of yellow halo was considered to be a positive response. Results on cellulolytic activity of all bacteria and fungi were determined in an identical manner by this method.

Presence of chitinolytic activity of bacteria and fungi was evaluated according to Arora et al (2005). The degree of enzymatic activity was assessed by measuring the radius of the clear zone appearing around each colony. Bacterial and fungal production of cellulases and chitinases was thus estimated qualitatively according to the scale:

- 0 no production
- + weak production with a clear zone <1 mm diameter

- ++ moderate production with a clear zone 2-3 mm for cellulase and 1-2 mm for chitinase
- +++ strong production with clear zone > 3mm for cellulase and > 2mm for chitinase activity.

Among cellulolytic and chitinolytic bacteria and fungi, some bacterial species have the ability to produce siderophores. They can compete for iron (Fe) causing iron depletion for the pathogens. To identify potentially beneficial effects of bacteria in terms of their ability to combat pathogens, bacterial fluorescence activity measurements were made on all bacterial strains which showed cellulolytic as well as chitinolytic activity. Kings medium B agar was used to assess the ability to produce fluorescent pigments according to King *et al.* (1954). After two days of inoculation, this activity was checked using UV light at 254 nm to identify fluorescent activity.

Effects on rice seed germination and seedling growth

To examine the direct effect of bacteria and fungi on rice seeds, 30 bacterial strains and 19 fungal isolates were chosen for inoculation of rice seeds. The rice seeds were purchased from the general market in Vietnam. Selection was made on the basis of different degrees of cellulolytic and chitinolytic traits. Each strain (10^8colony forming units, cfu/ml) was freshly cultured and suspended in 10 ml of sterile 0.01M $MgSO_4$ solution. Rice seeds were bacterized by immersing them in each suspension for one and a half hour. Fungal isolates were grown as stationary cultures on Hagen broth (HB) between 4 and 7 days depending on the isolate. The mycelial mats were harvested and ground aseptically with utmost care to provide a hyphal suspension without affecting the viability of the hyphae. Rice seeds were inoculated with each fungal suspension in the same manner as with bacteria. Control plates with either $MgSO_4$ solution or HB only were identically treated. The treated seeds were placed on sterile moist filter paper with 25 rice seeds per plate and two replicates for each isolate. All seeds were incubated at 25°C in the dark. Effects of bacteria on rice seeds were recorded as number of seeds germinated, and stimulation or inhibition of radicle length one week after incubation.

Molecular identification

<u>DNA extraction from fungal isolates</u>

CTAB buffer 2% (1Mtris-HCl (pH 8.0), 5.0 M NaCl, 0.5 M EDTA, and 2% cetyltrimethylammonium bromide) was used for DNA extraction. During the process, proteins including nucleases that destroy DNA and RNA as well as polysaccharides that inhibit some of the enzymes used in molecular biology were removed from DNA to get a high DNA yield. The purpose of this method is to get fungal DNA yields. Fungal isolates were grown on HB. Their mycelial mats were harvested and transferred to an Eppendorf tube of 1.5 ml and manually crushed in 1000µl of CTAB buffer and crushed carefully with a micropestle.

The mixture was incubated at 65° C for 1 hour and centrifuged at 13000 rpm for 10 minutes at 9500 RCF. The supernatant (700 µl) was mixed with 500 µl chloroform and centrifuged before transferring the upper phase (free from protein) to a new tube for precipitation of DNA by adding 1.5 volumes of cold isopropanol followed by centrifugation at maximun speed (13000rpm) for 20 minutes. The DNA pellet was washed with 70% cold ethanol (EtOH) and centrifuged at 6500 rpm for 5 minutes and left to dry overnight at room temperature (25°C). Finally, the pellet was dissolved in 100µl of milli-Q water and the DNA extract was stored at -20°C until PCR amplification was conducted. Regarding bacterial strains, pure colonies were used directly for running PCR by diluting them in milli-Q water. The DNA concentration of these suspensions was approximately 0.5 ng/µl. They were used in the PCR without further processing.

DNA amplification and sequencing

The polymerase chain reaction (PCR) is a method for oligonucleotide primer-directed enzymatic amplification of a specific DNA sequence of interest. PCR was performed using an Applied Biosystems GeneAmp PCR 2700. A total volume of 50 µl was used to amplify DNA extracts by a mixture of 0.2mM of all four nucleotides (dNTP), 10 µM of each primer (27f/ 907r (Noll *et al.*, 2005) for bacteria and ITS 1f/ ITS4 (Kennedy *et al.*, 2005)for fungi), 250 µM ThermoRed DNA polymerase and extracted DNA (DNA template).

The thermocycling program for bacteria started at 94°C for 3 min followed by 25 cycles at 92°C for 45 seconds, 50°C for 30 seconds, 72°C for 30 seconds and ended by a final 7 min extension at 72°C. The program for fungi started at 94°C for 5 min followed by 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds. The program was ended by a final 7 min extension at 72°C. The yield of PCR products was verified by separating them on a 1% D1- agarose gel at 150V for 40 min. The gels were stained in ethidium bromide for 15 min and rinsed in water for 15 min. Each gel was then photographed digitally and viewed in the programme Quality One® (Bio-Rad). Finally, the PCR products were precipitated with 70% cold ethanol and sequenced utilizing the AB1 big Dye Terminator technology on an AB1 3730 machine.

All isolates were characterized by sequencing of the 16S DNA for bacteria and ITS-region for fungi. Sequences were arranged into contigs according to their similarity by using DNASTAR software. Reference sequences were compared with published sequences in the Genbank database with the BLAST program. Sequences were aligned the Clustals W, the alignment was manually edited before the Neigbour-joining analysis was conducted using PAUP (Swofford,1998). The phylogenetic tree of the bacteria and fungi was evaluated with 100 bootstrap replicates.

Microcosm experiment to study the effect of biodiversity on rice straw decomposition

Rice straw was collected from the two fields after harvesting of rice seeds. The straw was dried at 60°C and stored at 22°C. Before setting up the long experiment, the straw was cut into approximately 5 cm pieces and its moisture content was adjusted to 60% in order to make the environment suitable for microbial growth.

A total of 10 bacterial and 6 fungal species with different functional characteristics were chosen for the experiment. They were inoculated singly or in different increasing numbers of combinations according to the experimental design presented in Appendix Table 9. The microorganisms were multiplied on the TSB and HB for bacteria and fungi respectively and their fresh inocula were prepared as described below.

The microcosms used for the experiment consisted of auctoclavable polyethylene bags (50 cm x 31 cm) containing straw (15 gram dry weight) and distilled water enough to give 60% moisture. The bag was closed with a cotton plug and autoclaved twice for one hour each. The inocula were prepared by suspending fresh cells of bacteria in TSB (1.5g/l) and blending fresh fungal mats in diluted HB (10%) respectively. The suspensions were diluted before aseptically inoculated to microcosms (5ml/microcosm) using as syringe and sealing the needle hole with tape. For different mixture inoculations, an equal amount of each isolate was added to make 5ml/microcosm.

The control microcosms were arranged in a similar manner using microbe-free culture media. Incubation of all microcosms was carried out by placing them spaciously in a chamber (a light/ dark regime of 12/12 hours) at 30°C for 6 weeks. The moisture content of the rice straw in the microcosms was adjusted to 60 % during the whole experimental period by adding SDW whenever necessary. After six weeks, the rice straw was harvested and dried over night in an oven at 60°C. The biomass from each microcosm was calculated and transformed to % weight loss compared with the weight at the beginning of the experiment. Functional dissimilarity was calculated as the difference between % weight losses of monocultures in species combinations divided with the number of possible interactions between species in a microcosm.

Statistical analysis

Chi^2 tests were used to evaluate differences in functional traits and phylogenetic composition among the two soils tested. Shannon-Weaver diversity indexes (H´) were calculated for the two sites based on the presence and abundance of taxonomic families of bacteria and fungi (Figure 3) as well as their functional groups (Figure 1) according to the equation $H' = -\sum Pi \log Pi$ (Eichner et al., 1999). The term Pi was calculated as follows: $Pi = ni/N$, where ni is the

number of individual functional group or the isolate and N is the sum of the total functional groups or families.

Interactions between functional characteristics of cellulolytic and chitinolytic activity were assessed by simple regressions between activity and infection, radicle length and shoot growth. The relationships between number of species, functional groups and functional dissimilarity on the one hand and % weight loss on the other hand were evaluated by single regressions as well as with multiple regression under a general linear model. The effect of number of species, functional groups, functional dissimilarity as well as fungi, bacteria and all individual species were evaluated in an analysis of multiple regressions under a general linear model.

Results

Soil characteristics of the rice fields

The soil environment at the time of sampling was different at the two sites with respect to moisture conditions. The water level at Hoa An (HA) was about 30 cm above the soil surface while the soil at Vinh Nguon (VN) was dry. The soils also differed in most of the chemical variables investigated. Compared to the VN soil, the HA soil was acidic with comparatively low amounts of exchangeable Ca and total K, but with high amounts of organic carbon and available P ($P_{Bray\ I}$) (Table 1).

Table 1: Soil parameters of two rice fields.

Site	K_{tot} (%K)	SOM (%C)	N_{tot} (%)	P_{tot} (% P)	$P_{Bray\ I}$ (mgP/kg)	Ca_{ex} (meq/100g)	pH_{H2O} (1:2,5)
Hoa An soil	1.68	8.15	0.45	0.04	5.2	1.88	3.64
V.Nguon soil	1.94	2.99	0.25	0.03	1.3	11.6	5.02

Rice stubble associated microorganisms and their functional characteristics

A total of 259 bacterial strains (144 bacterial strains from the VN site and 115 from the HA site) and 45 fungal isolates (22 from the VN site and 23 from the HA site) were obtained with the isolation method used. The isolated microorganisms varied in morphological characteristics (e.g. floating vs non-floating), growth patterns (e.g. colour and form of the colony type) and rate of growth (slow growing vs fast growing, empirical data not recorded). None of the bacterial strains proved to be fluorescent.

Though all isolations were made on cellulose amended media, on evaluation of their activity some isolates were not cellulolytic. The majority of the bacteria, 52% (134 strains) lacked any enzymatic activity compared to only 7% (3 isolates) among the fungi. Results regarding the functional characteristics of all bacterial and fungal isolates are summarized in Table 2. The level of enzyme activity was different between isolates. Of all the bacteria tested, 41% were cellulolytic and 8% exhibited both cellulolytic and chitinolytic activity. In comparison 24% of the fungi were cellulolytic and 51 % exhibited both activities. 18% of the fungi isolated were chitinolytic and all of these originated from the VN site.

Table 2: Distribution of rice stubble associated with cultivable fungi and bacteria based on their origin and two functional characteristics.

Sample	Vinh Nguon soil			Hoa An soil		
	+++	++	+*	+++	++	+
Cellulolytic activity						
Bacteria (105/259)	10	10	41	1	20	23
Fungi (11/45)	-	1	4	1	2	3
Chitinolytic activity						
Fungi (8/45)	-	1	7	-	-	-
Both cellulolity and chitinolytic activity						
Bacteria (20/259)	-	-	2	6	8	4
Fungi (23/45)	-	2	5	-	6	10

- * degree of hydrolytic activity expressed as +++ strong, ++ moderate, + presence and –absence
- Bacteria were isolated on diluted tryptic soybroth agar and fungi were isolated on Hagen agar, both amended with cellulose.

The proportions of different functional groups in rice straw originating from the VN site and the HA site are presented in Figure 1. At the VN site, out of 144 bacterial strains, 61 strains exhibited cellulolytic activity and two showed both cellulolytic and chitinolytic activity, whereas, 44 out of 115 bacterial strains isolated from the HA site showed cellulolytic activity, and 18 strains produced both cellulases and chitinases. There were significant differences in bacterial functional groups between the two field sites (Chi^2 $p<0.01$; Shannon Weaver test in Appendix 5), owing to the number of cellulolytic plus chitinolytic bacterial strains (Chi^2 $p<0.05$) but not to the isolates with cellulolytic activity (Chi^2 $p>0.05$) (Figure 1a and1b).

At the Vinh Nguon site, out of 22 fungal isolates 5 had cellulolytic activity, 8 showed chitinolytic activity and 7 exhibited both cellulolytic and chitinolytic activity. There were 23 fungal isolates identified from the Hoa An site, 6 of which showed cellulolytic activity. No fungus showed chitinolytic activity only. 16 fungal isolates showed both cellulolytic and chitinolytic activity. The two soil types differed significantly only with regard to the number of chitinolytic fungi isolated (Chi^2 $p<0.05$) and bi-enzymatic activity (Chi^2 $p<0.01$) (Figure 1c and 1d). There was no difference in fungal functional groups between the two sites (Chi^2 $p>0.05$; Shannon Weaver index in Appendix 6).

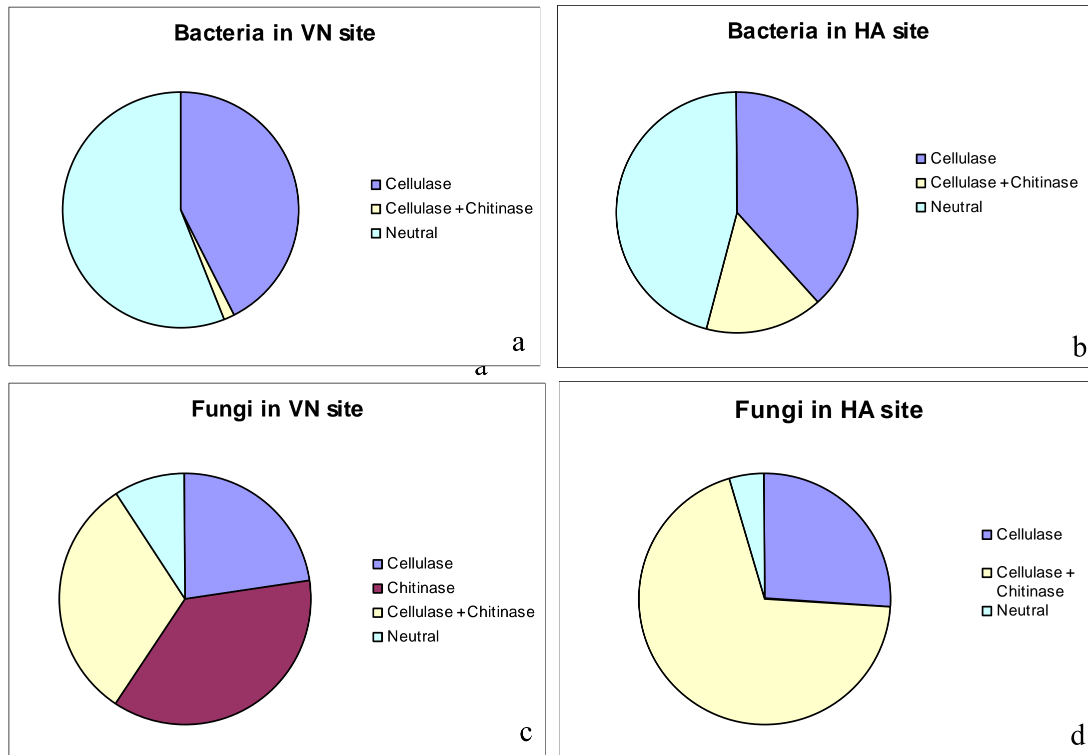

Figure 1: The proportion of different cultivable bacterial (Fig 1a and 1b) and fungal (Fig 1c and 1d) functional groups in rice straw originating from Vinh Nguon (VN) soil and from Hoa An (HA) acidic soil

Effects of stubble associated microorganisms on rice seed germination

Among a total of 49 isolates tested (30 bacteria + 19 fungi) from different families, both beneficial and deleterious effects were measured (Appendix 2 and 3). Based on the effect of microbes on germination and on radicle length compared with the corresponding controls, the results showed that members of *Bacilliaceae* and *Enterobacteriaceae* had either positive or negative effects on rice seed emergence/ radicle length. Only 7 bacterial strains seemed to exhibit a beneficial effect in terms of stimulated radicle length and maximum number of seeds emerged. They were *Bacillus* sp., *Azospirillum, Stenotrophomonas* sp., *Citrobacter* sp., *Bacillus cereus, Pantoes dispersa* and one unidentified bacterium. While almost three times more strains (22) were apparently deleterious. The deleterious strains belonged to *Bacillus pumilus, Serratia* sp., *Pantoea dispersa* and *Burkholderia gladioli, Herbasiprillum sp.* (Appendix 4). Interestingly, *Pantoea dispersa* exhibited both beneficial and deleterious effect. In comparison, most fungal isolates seemed to be harmless to rice seed germination

(Appendix 3). In general, the effect of fungi on rice radicle length was more positive than the effect of bacteria. Effects on rice seed germination and radicle length growth were empirically recognized. However, no statistical correlation was found between the bacterial cellulolytic and chitinolytic activity and the effect on rice seedling growth. Neither was there any correlation between fungal enzymatic traits and germination nor radical length (Figure 2). However, there was a positive correlation between seed colonization ability of fungi and their cellulase trait (r= 0,74; p< 0,01) but a negative correlation of fungal chitinase trait and rice seeds colonized (r= -0,45; p< 0,01).

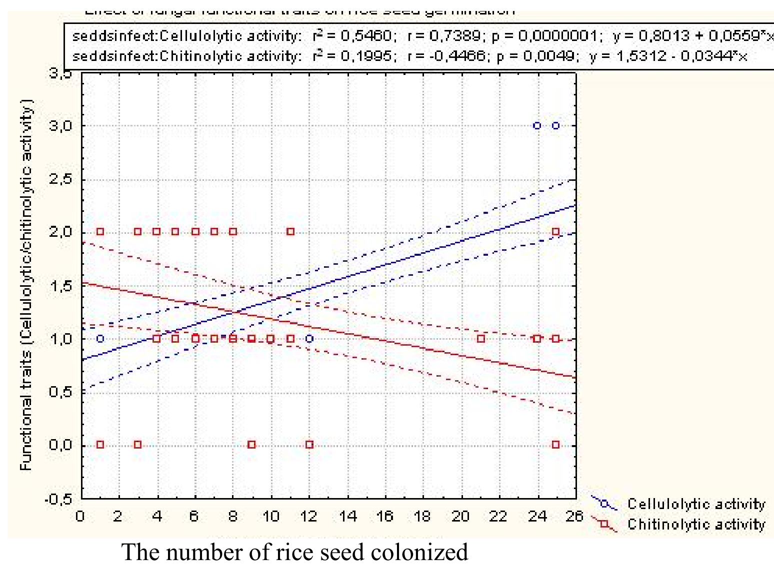

Figure 2: The correlation between fungal functional traits and rice seeds colonized

Family	Total no. of isolates	VN soil (No. isolates)			HA soil (No. isolates)		
		Total	Cellulolytic	Both activities	Total	Cellulolytic	Both activities
Bacteria							
Bacillaceae	54	40	36	2	14	9	2
Burkholderiaceae	55	21	2	0	34	2	3
Caulobacteraceae	1	0	0	0	1	1	0
Enterobacteriaceae	50	24	19	0	26	17	5
Flavobacteraceae	1	0	0	0	1	0	0
Flexibacteraceae	4	0	0	0	4	0	4
Microbacteriaceae	12	3	3	0	9	6	0
Moraxellaceae	5	4	0	0	1	0	0
Neisseriaceae	4	0	0	0	4	0	0
Oxalobacteraceae	6	0	0	0	6	3	1
Paenibacillace	1	0	0	0	1	1	0
Pseudomonadaceae	47	42	1	0	5	0	1
Rhizobiaceae	1	0	0	0	1	1	0
Rhodospirillaceae	3	0	0	0	3	3	0
Sphingomonadaceae	1	0	0	0	1	1	0
Staphylococcaceae	1	1	0	0	0	0	0
Xanthomonadaceae	13	9	0	0	4	0	2
Total	259	146	61	2	115	44	18
Fungi							
Ceratobasidiaceae	1	0	0	0	1	0	0
Hypocreaeceae	2	2	0	2	0	0	0
Mucoraceae	9	9	0	2	0	0	0
Nectriaceae	15	5	3	2	10	0	10
Pleosporaceae	3	0	0	0	3	3	0
Sordariaceae	4	4	1	0	0	0	0
Sporormiaceae	7	0	0	0	7	2	5
Trichocomaceae	1	1	1	0	0	0	0
Uncultured fungi	3	1	0	1	2	1	1
Total	45	22	5	7	23	22	16

Table 3. Phylogenetic distribution of cultivable bacterial and fungal families found in rice stubbles sampled from The Vinh Nguon soil and the Hoa An acid soil in Mekong Delta, Vietnam.

Phylogenetic distribution of microorganisms associated with rice stubbles

To resolve the relationships of the cultivable bacteria and fungi in the rice stubbles, all bacteria and fungi were sequenced. All bacteria and fungi were identified by sequencing. All results from the sequence analyses are summarized in Table 3 and Figures 4 and 5.All the identified isolates were organized in contigs that belonged to different families (Table 3). Bacteria and fungi with cellulolytic or chitinolytic activities were found to be present in diverse families as well. A total of 17 bacterial families and 9 fungal families were identified. The bacterial community of the acid HA site harboured 16 families and was significantly different in composition (Chi^2 $p<0.01$ and Shannon Weaver index (Appendix 7)) compared to the bacterial communities in the VN site. The latter harboured 8 families with a few numbers of isolates within the families (Figures 3a and 3c). Further more, bacteria and fungi with cellulolytic and chitinolytic activities were found to be present in diverse families.

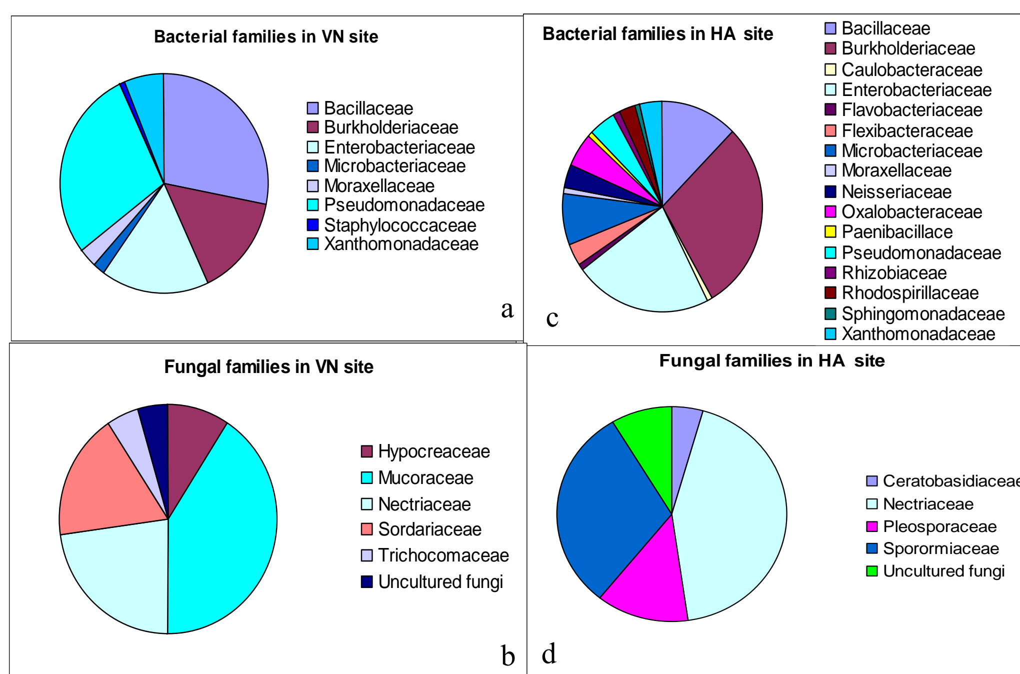

Figure 3: The proportion of different groups of bacterial and fungal families in rice subbles originating from VN site (a and b) and from HA site (c and d)

Approximately 62% of all isolates belonged to Bacillaceace, Burkholderiaceae and Enterobacteriaceae that dominated both soils. Pseudomonadaceace was also common, 18% of all strains belonged to this family. A higher number of strains in this family were present in the VN soil than in the acid HA soil. Results in Table 3 and Figure 4 and Figure 5 showed the distribution of cellulolytic and both cellulolytic and chitinolytic bacteria and fungi in stubble from two fields.

Bacillaceae and Enterobacteriaceae contained more bacterial contigs with cellulolytic activity in both soils than other families. Some bacterial families e.g. Rhizobiaceae, Sphingomonadaceae, Caulobacteriaceae, and Rhodospirillaceae were related to nitrogen fixing bacteria and they too showed cellulolytic activity. These particular families were detected only in stubbles from the acid soil.

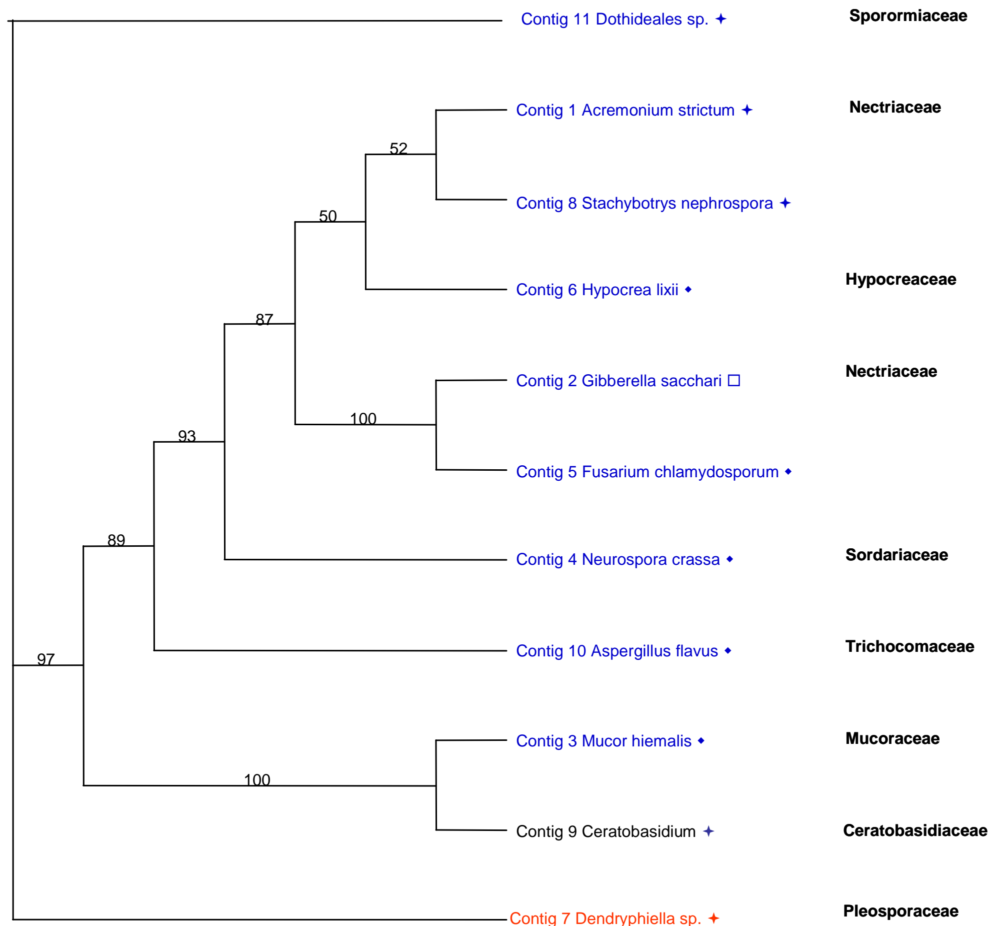

Figure 4. Fungi: Unrooted Phylogenetic tree based on a Neighbor-joining search. Numbers on branchces indicate bootstrap support based on 100 replicates. Contigs which include isolates with cellulolytic activity are shown with red text while contigs with combined cellulolytic and chitinolytic activity are shown in blue text. Symbols indicate site of isolation ◆ for VN ✦ for HA and ☐ for present in both.

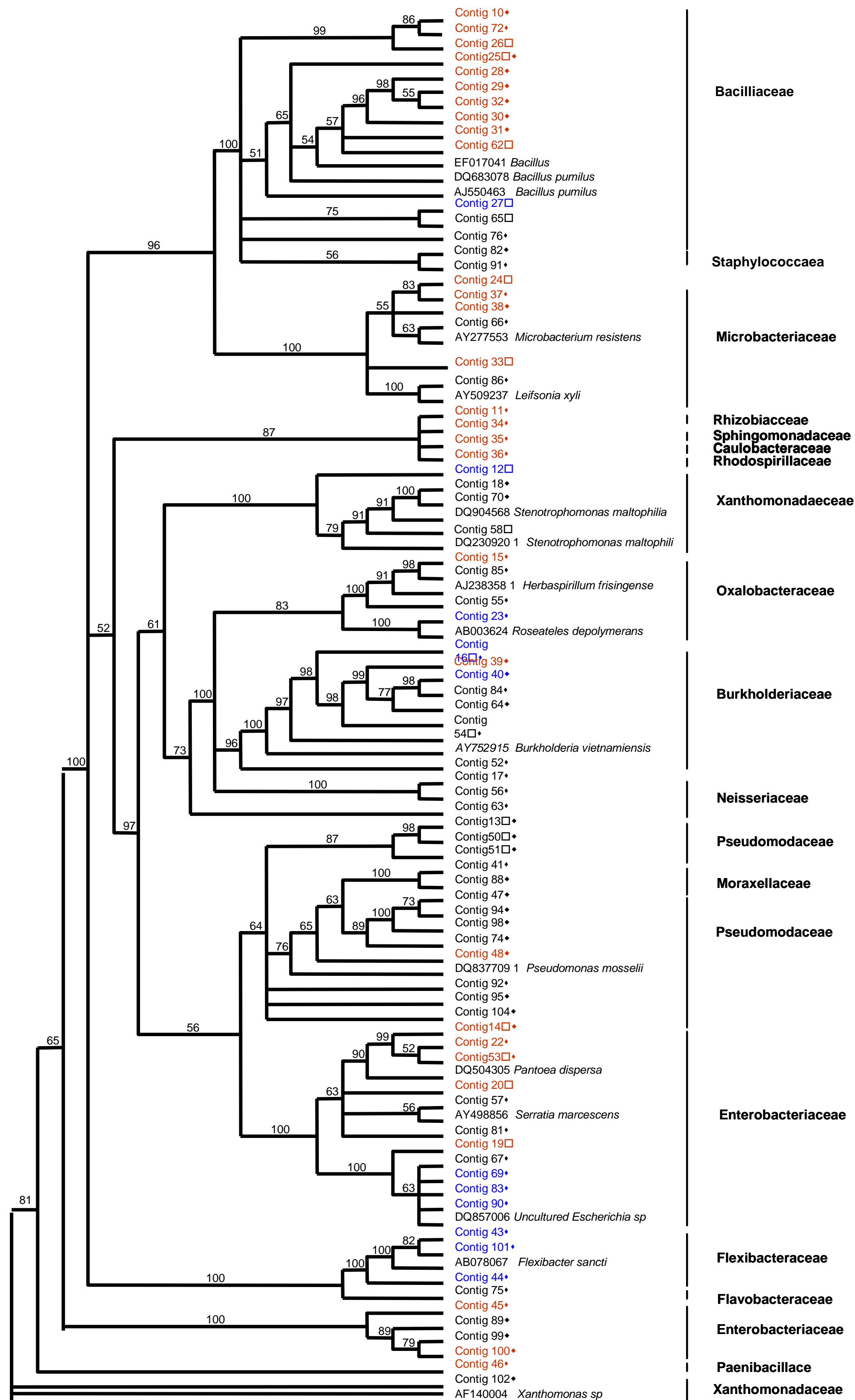

Figure 5. As in Figure 4 for Bacteria

A total of 9 fungal families were found belonging to 11 different contigs. The number of the fungal families did not differ between the two rice fields (Chi^2 $p>0.05$ or by Shannon Weaver index (Appendix 8)) (Figure 3c and 3d). The family Nectriaceae was dominant in stubbles from both fields. Within Nectriaceae, the functional characteristics were more diverse than those among members of other families in both sites (Fig 3 and 5)**.** The fungal families, Mucoraceae, Hypocreaceae, Sordariaceae appeared only in the Vinh Nguon site while Pleosporaeae, Ceratobasidiaceae and Sporomiaceae were present only in the Hoa An site.

Identification from DNA sequences further revealed identification of some interesting strains belonging to of *Bacilus cereus, Burkholderia gladioli, B. cepacia* that are known potential rice pathogens. *Actinetobacter sp., Serretia marcescens* are known to be opportunistic human pathogens, these were also isolated from stubble. Some nitrogen fixing strains belonged to the Rhizobiaceae, or free nitrogen fixation (*Sphingomonas sp.* from Rhodospirillaceae*, Herbaspirillum sp.* species belonging to the Oxalobacteriaceae as well as several *Burkholderia* species related to *B. Vietnamese)***.** *Bacillus subtilis*, commonly considered as a biological control agent in horticulture and agriculture, was also identified.

Effect of microbial biodiversity on straw decomposition in microcosms
After 6 weeks of inoculation, the straw inoculated with either single cultures

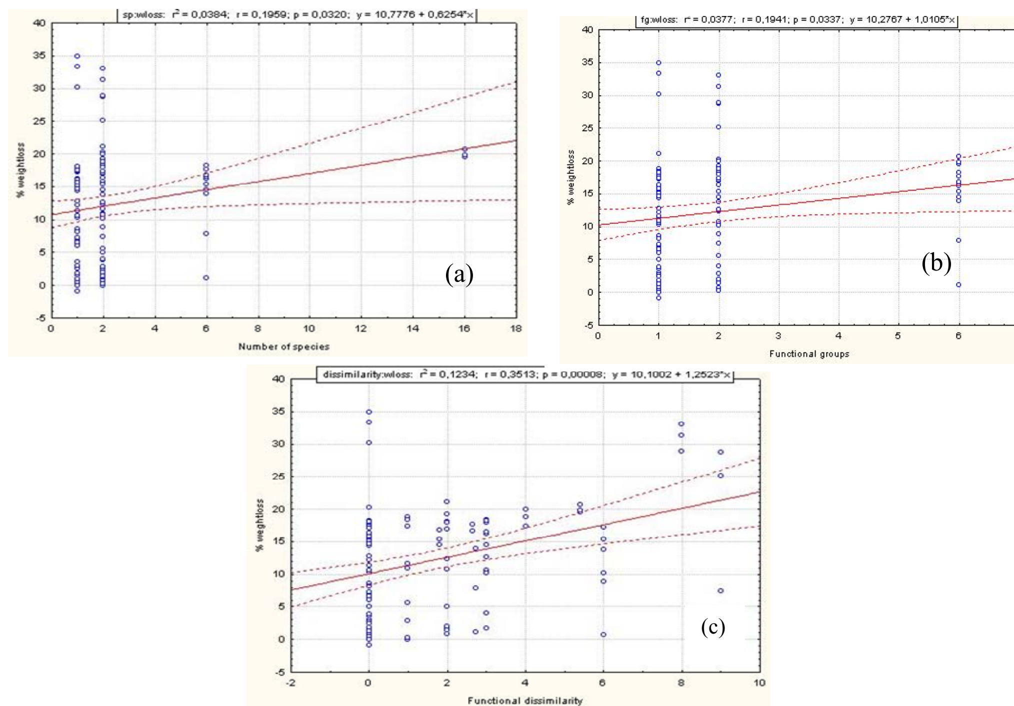

Figure 6. Number of species (a), functional groups (b) and functional dissimilarity (c) of microorganisms in relation to the rice straw weight loss.

or in various mixed combinations in microcosms exhibited a weight loss, measured as dry weight, up to 32% depending on the treatment (Figure 7). There was a positive regression between the number of species inoculated and weight loss (r^2= 0.0384, $p<0.05$). There was a weak positive relationship between the number of functional groups and weight loss (r^2= 0.0377, $p<0.05$) as well as between functional dissimilarity and weight loss (r^2=0.1234, $p<0.01$) (Figure 6).

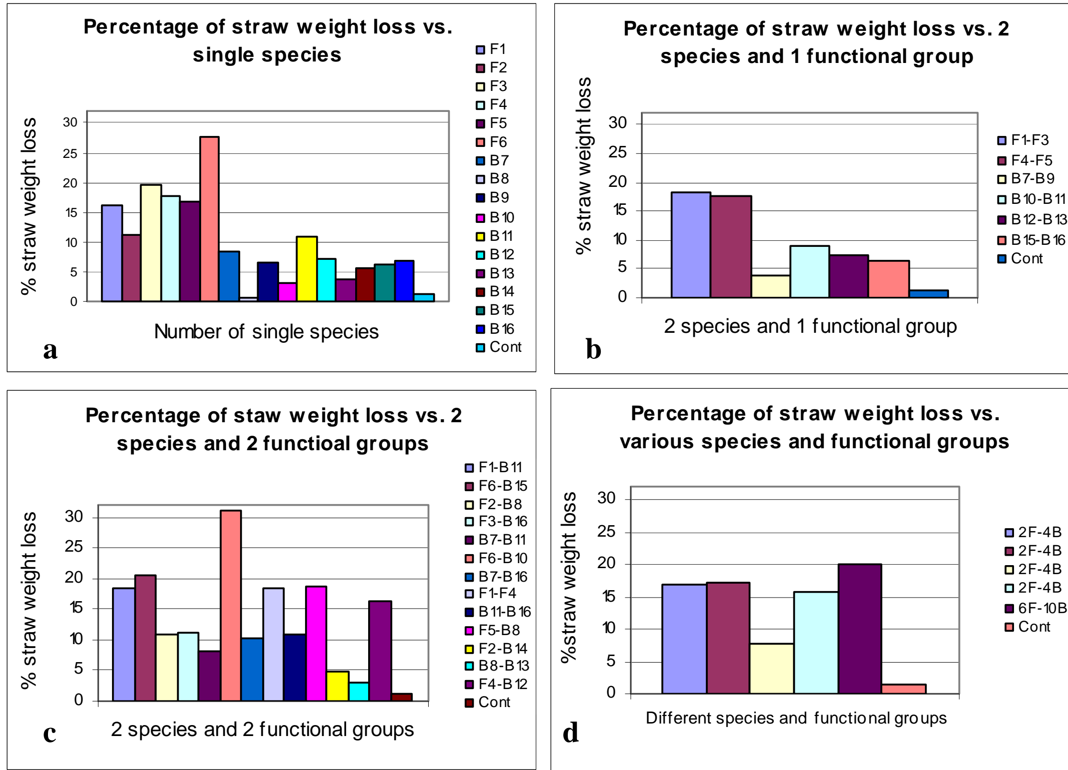

Figure 7. Effect of the number of species and functional groups on the percentage of straw weight loss

Multiple regressions under a general linear model were used to infer if the functional diversity response was due to single species effects or facilitative interactions. The results infer that both combinations of species and dissimilarity contribute to decomposition as well as the individual fungal species and one of the bacterial species (Table 4).

Table 4: Multiple regression analyses between five individual fungal species and one bacterial species and functional groups in combination with the number of species to explain the general diversity effect**.**

	df	Mean Square	F-value	P
Interaction	1	1905.93	60.93	0.001
Bacteria	1	272.83	8.72	0.01
Species	1	1132.71	36.24	0.001
Dissimilarity	1	303.59	9.71	0.01
Fungus 1	1	370.03	11.83	0.001
Fungus 3	1	220.95	7.064	0.01
Fungus 4	1	560.97	17.93	0.001
Fungus 5	1	1092.47	32.91	0.001
Fungus 6	1	1096.18	35.04	0.001
Bacterium 11	1	291.03	9.304	0.01
Error	110	31.28		

The percentage of straw weight loss in treatments with single fungal species ranged between 12 and 28 % whereas the percentage loss was only 0.6 - 11 % in treatments inoculated with bacteria (Figure 7a). Interestingly, the highest straw weight loss (28%) compared to all other single inoculants was observed in the microcosms where *Stachybotrys* sp. was inoculated. singly or as a co-inoculant. Straw inoculated with Dothidales resulted in the lowest percentage weight loss among fungi but this loss was still higher than the loss obtained in any of the single bacterial treatments.

The percentage straw weight loss ranged between 4% and 19% in two-species combinations with species from the same functional group (Figure 7b). The weight loss in the presence of two fungal species was twice as high as that obtained by two bacterial species. The weight loss due to combined treatments with two species and two functional groups differed between 3% and 32%. In these microcosms, the combination treatments with *Stachybotrys* sp. showed a higher weight loss than treatments with other combined inocula (Figure 7c). The resulting straw weight losses with 6 species + 6 functional groups and 16 species + 6 functional groups are shown in Figure 7d. The treatment carrying all 16 species resulted in a higher weight loss (21%) than all other combined treatments (8-16%).

Discussion

The comparison of microbial diversity between two different rice fields is highly interesting since earlier studies of microbial communities in rice fields have typically been performed on single soils. The bacterial community in the Hoa An soil was much more diverse than that in the Vinh Nguon soil. Regarding fungi, only isolates representing Nectriaceae representatives were common in both fields. Other fungal families appeared either in the Hoa An soil or in the Vinh Nguon soil. This result agrees well with the results of Kennedy *et al.* (2005) who studied microbial communities in grasslands and found that bacterial ribotype numbers were significantly affected by soil chemical properties while fungal ribotype numbers were significantly affected by grassland type. One difference between the soils used in this study was the moisture content at sampling, which was much higher at the Hoa An site than at the Vinh Nguon site. Furthermore, soil chemical variables indicated important site differences (Table 1). The Hoa An soil had lower values of pH and exchangeable calcium but higher total nitrogen, organic carbon and available phosphorus than the Vinh Nguon soil. Similarly to my study, Øvreås and Torsvik (1998) found that sandy soil with low organic matter content showed a lower diversity of cultivated bacteria than organic soil. On the other hand Johnson *et al.* (2003) found that pH and organic carbon content did not show any relations with the bacterial community structure in agricultural soils.

Functional traits of micro-flora among stubble from two soil fields

The functional diversity in terms of enzymatic activities of bacteria and fungi varied among the two sites. Both cellulolytic and chitinolytic activity have been suggested to be involved as the mechanisms used by biocontrol agents for suppression of plant pathogens. Arora *et al.* (2005) studied the microbial activity in composts and found that bacteria with cellulolytic activity were able to inhibit *Rhizoctonia solani* causing damping off and that fluorescent siderophore production was positively related to the antagonism against another pathogen *Pythium sp*. We observed both cellulolytic and chitionlytic activities in members from specific families (Figures 4 and 5).

The sequencing results showed that Baclliaceae, Burkholderiaceae, Enterobactericeae and Pseudomonadaeceae were dominant in both fields and the majority of the isolates in those families were present in the two fields. These results agree with results from Japanese rice paddy fields which also showed that these families were abundant in the field. Rice fields could thus be a good environment for their growth.

The family of Burkholderiaceae contains over 30 species that occupy a remarkably diverse range of ecological niches ranging from contaminated soil to the respiratory tract of humans (Coenye and Vandamme 2003). The species *Burkholderia vietnamensis* is capable to fix

nitrogen from the atmosphere. *B. kururiensis* was isolated from an aquifer polluted with trichloroethylene (Zhang *et al.*, 2000). Some *Burkholderia* species such as *B. gladioli* and *B. cepacia* are believed to be pathogenic (Coenye and Vandamme 2003). Enterobactericeae consist of strains with both pathogenic and beneficial potential while *Serretia marcescens* is an opportunistic pathogen occurring in humans (Dauga 2002). My results concerning this family showed that there were two branches of Enterobactericeae present in the phylogenetic tree. This agrees with the finding of Dauga (2002) who identified that *Serratia* species formed a monophyletic group that was validated by significant bootstrap values. The *Klebsiella* and *Enterobacter* genera seem to be polyphyletic in this study, but the branching patterns of *gyr B* and 16S rDNA gene trees were not congruent (Dauga 2002). The Caulobacteriaceae, Flavobacteriaeceae, Flexibacteriaceae, Meisseriaceae, Oxalobacteriaceae, Paenibacillaceae, Rhizobiaceae, Rhodospirillaceae, and Sphigomonadaeceae families were present in the HA soil. They may have been present due to the fact that the Hoa An soil was saturated with water after the harvesting of rice grain. Therefore, they dominated in this field. According to Holmes (1992), species belonging to *Sphingomonas* and the CFB group (*Cytophaga-Flavobacterium- Bacteroides)* and *Chryseobaeterium (*Flavobacteriaceae*)* are common inhabitants of aquatic environments. *Sphingomonas* sp. is considered to be adapted to oligotrophic and starvation conditions (Balkwill *et al.*, 2003). This result was similar to the results from floodwater of a Japanese paddy field. The bacterial communities in the floodwater were supposed to include floodwater-specific members as well as members associated with other habitats in the paddy fields (Nakayama *et al.*, 2006).

Members of the Rhizobiaceae family are capable of fixing atmospheric nitrogen and hence could promote plant growth in nitrogen limited systems given that plant growth is nitrogen limited (Steenhoudt and Vanderleyden, 2000). They are often found in the rhizosphere of different plants. Interestingly, they were found in the rice stubble in this study.
Hypocrea lixii is the sexual state of *Trichoderma harzianum.* It is considered to have properties suitable for the biological control of plant pathogenetic fungi (Chaverri *et al.* 2002). Also, some fungi belonging to Nectriaceae were potential pathogens of rice in the developing rice period and *Aspergillus flavus* was a potential pathogen in the post harvesting period.

Effect of microbial biodiversity on straw decomposition in microcosms

In earlier studies in which biodiversity has been linked to ecosystem functioning species diversity has been considered to also represent the functional diversity (Diaz and Cabido 2001; Loreau *et al.*, 2003). To date, the majority of studies on microbial diversity has focused on either the responses of individual species (de Bello *et al.*, 2005) or on community –level traits ("aggregated traits" sensu Garnier *et al.*, 2004). Few researchers have attempted to link these two levels of response (but see McIntyre and Lavorel 2001), although the use of traits and their distribution within communities has been recognized as a promising means for understanding mechanisms of community assembly in order to predict community dynamics (Diaz *et al.*, 1999; McGill *et al.*, 2006). In my study I found that there were positive relationships between number of species inoculated and rice straw weight loss. This agrees well with previously published results on biodiversity/ decomposition relationships among bacteria (Wohl *et al.*, 2004). The results reported by Setälä and Mclean (2004) and Tiunov and Scheu (2005), who made fungal diversity - decomposition experiments, revealed that there was a positive effect of fungal species richness on decomposition at relatively low diversity values but no further influence beyond an actual diversity of 5 to 10 fungal taxa. Studies concerning boreal wood decaying fungi actually showed a negative relationship between species diversity and decomposition. It has been shown that the competition is very intensive among wood decaying fungi, and a competition associated with a high energetic cost may explain the found negative relationship between number of species and competition in this system (Toljander *et al.*, 2006).

The positive relationship between species diversity and straw decomposition in my study may be explained by either facilitative interactions among species or simply by a statistical effect: The presence of several high performance species is much more likely to occur in high diversity than in low diversity communities. However, the results reveal that in addition to a general effect of an increased species and functional diversity, five fungal and one bacterial species significantly affected the decomposition rate although they were present in different functional groups from high cellulolytic to low celllulolytic activity for fungi and both celllulolytic and chitinolytic activity for bacterial species. Therefore, it is likely that both facilitative interaction and species effects contributed to the positive relationship between species/ functional biodiversity and rice straw decomposition.

Rice cultivation is one of the most important activities in Vietnamese agriculture. The intensive rice cropping systems may cause many changes in the soil environment due to burning of rice straw, decreasing soil nutrients resulting from non-compensated rice uptake and an increase in soil pathogens due to repeated growing of the same crop in the same fields.

The microbial flora of rice fields has so far not been extensively studied in tropical and subtropical areas of the world where rice is predominantly grown. Therefore, understanding the microorganism community structure in the rice field is essential to maintain soil fertility as well as rice production. This study shows that there are differences among rice field soils both in terms of phylogenetic and functional microbial diversity. Furthermore, an interesting positive relationship between microbial diversity and rice straw decomposition was found. For the future, further studies of the microbial community in South East Asian rice fields will be interesting. Obviously, the knowledge of the uncultured part of the microbial community will also be of great interest including the Archaea community structure including short-term temporal variation after harvesting and the effect of degree of flooding. From a rice production perspective, further knowledge about mutualistic symbionts such as arbuscular mycorrhizal fungi and nitrogen fixing bacteria as well as nonsymbiotic beneficial organisms may be crucial for maintaining a high rice production. Time series studies of the microbial successional patterns compared between high intensity rice cultivation and different crop rotation management strategies may provide further insight into the microbial ecology of these farming systems.

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Appendices

Appendix 1: Summary of bacterial contigs identified in the rice stubble from two fields

				Vinh Nguon soil			Hoa An soil		
Contig	Family	Genus	Species	Identify	Cellulase	Chitinase	Identify	Cellulase	chitinase
10	Bacillaceae	Bacillus	subtilis	162	3	0			
25	Bacillaceae	Bacillus	pumilus	81 95 96 101 102 105 113 114 159 191 215 216 217 219 227 228 231 232 255 256 274	1 1 1 1 1 1 1 1 1 1 2 3 2 3 1 1 2 3 1 3 1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	70 146	1 1	0 3
26	Bacillaceae	Bacillus	subtilis	165 276 277	3 3 3	0 0 0	157 158 248	2 2 2	0 0 0
27	Bacillaceae	Bacillus	cereus	60 61	2 2	1 1	67 249	2 2	2 0
28	Bacillaceae	Bacillus	pumilus	82 264	1 3	0 0			

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
29	Bacillaceae	Bacterium	YC-4	214	2	0			
30	Bacillaceae	Bacillus	pumilus	85 90	1 1	0 0			
31	Bacillaceae	Bacterium	Sp.	265	3	0			
32	Bacillaceae	Bacterium	YC-4	213	2	0			
62	Bacillaceae	Bacillus	Sp.	59 97 104 254	2 1 0 1	0 0 0 0	69 120 121	1 2 2	0 0 0
65	Bacillaceae	Bacillus	Sp.	112 163 169	0 0 0	0 0 0	195	0	0
72	Bacillaceae	Bacillus	Sp.				145	2	0
76	Bacillaceae	Bacillus	Sp.				23	0	0
91	Bacillaceae	Bacillus	Sp.				252	0	0
16	Burkholderi aceae	Burkholde ria	gladioli	192 230	1 1	0 0	5 68 118 119 141 142 143 144	1 3 1 0 0 1 0 0	0 2 0 0 0 1 0 0
39	Burkholderi aceae	Burkholde ria	cepacia	289	1	0			
40	Burkholderi aceae	Burkholde ria	gladioli				33	1	2

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
49	Burkholderiaceae	Burkholderia	gladioli	76 77 79 204 205 222 260 285 286 302	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	3 4 13 18 20 25 27 35 36 184 186 199 203 237 281 303	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
52	Burkholderiaceae	Burkholderia	kururiensis				148	0	0
54	Burkholderiaceae	Burkholderia	gladioli	299	0	0	250 280 283	0 0 0	0 0 0
61	Burkholderiaceae	Burkholderia	cenocepacia	206 220 261	0 0 0	0 0 0			
64	Burkholderiaceae	Burkholderia	cepacia	229 287	0 0	0 0			
68	Burkholderiaceae	Burkholderia	cepacia	288	0	0			
71	Burkholderiaceae	Burkholderia	gladioli				275	0	0
77	Burkholderiaceae	Burkholderia	Sp.				282	0	0
78	Burkholderiaceae	Burkholderia	gladioli				37	0	0
79	Burkholderiaceae	Burkholderia	cepacia	193	0	0			

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
80	Burkholderiaceae	Burkholde ria	gladioli				253	0	0
84	Burkholderiaceae	Burkholde ria	gladioli				72	0	0
35	Caulobacteraceae	Caulobact er	Sp.				16	1	0
14	Enterobacteriaceae	Pantoea	dispersa	51 88 89 91 103 108 109 166 167 168 226 290 291 292 293 294 295	1 1 1 1 2 1 1 2 2 1 1 1 1 1 1 1 1	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	38 42 181 185 235 240 241 242	2 2 3 2 2 2 2 2	0 0 0 0 0 0 0 0
19	Enterobacteriaceae	Pectobacte rium	chrysant hemi	49	0	0	64	2	0
20	Enterobacteriaceae	Pantoea	agglome rans	53	0	0	153	1	0
22	Enterobacteriaceae	Entobacter ia	bacteriu m				180	2	0
45	Enterobacteriaceae	Serratia	marcesc ens				24	2	0

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
53	Enterobacteriaceae	Pantoea	dispersa	136 234	0 1	0 0	134 236 243 278	1 1 1 1	0 0 0 0
57	Enterobacteriaceae	Serratia	marcescens				30 127 154 183	1 0 1 0	3 0 3 0
67	Enterobacteriaceae	Enterobacter	Sp.				196	0	0
69	Enterobacteriaceae	Uncultured Escherichia	Sp.				32	2	1
81	Enterobacteriaceae	Enterobacteria	Sp.				41	0	0
83	Enterobacteriaceae	Citrobacter	Sp.				39	3	3
89	Enterobacteriaceae	Klebsiella	pneumoniae	297	0	0			
90	Enterobacteriaceae	Enterobacter	Sp.				65	1	3
96	Enterobacteriaceae	Enterobacter	Sp.				304	1	0
99	Enterobacteriaceae	Enterobacter	Sp.	273	0	0			
100	Enterobacteriaceae	Uncultured Enterobacter		115	1	0			
75	Flavobacteriaceae	Chryseobacterium	Sp.				12	0	0
43	Flexibacteraceae	Chitinophaga	Sp.				179 197	3 2	2 1
44	Flexibacteraceae	Flexibacter	sancti				22	2	1
101	Flexibacteraceae	Flexibacter	sancti				147	3	2
24	Microbacteriaceae	Microbacterium	laevaniformas	54	1	0	10 17 129 134	1 1 1 1	0 0 0 0
33	Microbacteriaceae	Clostridium	Sp.	98	1	0	40	2	0

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
38	Microbacteriaceae	Microbacterium	Sp.	99	1	0			
37	Microbacteriaceae	Microbacterium	laevaniformans				132	1	0
66	Microbacteriaceae	Microbacterium	Sp.				11 26	0 0	0 0
86	Microbacteriaceae	Leifsonia	xily				151	0	0
41	Moraxellaceae	Acinetobacter	Sp.				122	0	0
42	Moraxellaceae	Acinetobacter	Sp.	117	0	0			
60	Moraxellaceae	Acinetobacter	Sp.	189 190	0 0	0 0			
88	Moraxellaceae	Acinetobacter	Sp.	187	0	0			
17	Neisseriaceae	Chromobacterium	Sp.				123 124	0 0	0 0
56	Neisseriaceae	Chromobacterium	Sp.				19 28	0 0	0 0
15	Oxalobacteraceace	Herbaspirillum	Sp.				135 155 156	1 1 1	0 0 0
23	Oxalobacteraceace	Roseateles	depolymerans				73	3	3
55	Oxalobacteraceace	Herbaspirillum	frisingense				6	0	0
85	Oxalobacteraceace	Herbaspirillum	Sp.				21	0	0
46	Paenibacillaceae	Paenibacillus	Sp.				1	2	0
13	Pseudomonadaceae	Psedoumonas	mosselii	44 45 46 47 48 52 53 112 139 140	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	182	1	2

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
47	Pseudomonadaceae	Pseudomonas	putida	110	0	0			
48	Pseudomonadaceae	Pseudomonas	mosselii	111	1	0			
50	Pseudomonadaceae	Pseudomonas	plecoglossicida	56 58 62 86 87 92 137 138 164 170 207 208 212	0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0 0 0	125	0	0
51	Pseudomonadaceae	Pseudomonas	otitidis	171 174 175 194 268 269 270 300 301	0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0			
74	Pseudomonadaceae	Pseudomonas	Sp.	178	0	0			
92	Pseudomonadaceae	Pseudomonas	Sp.				133	0	0

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
93	Pseudomonadaceae	Pseudomonas	Sp.	263	0	0			
94	Pseudomonadaceae	Pseudomonas	putida	71	0	0			
95	Pseudomonadaceae	Pseudomonas	Sp.	221	0	0			
97	Pseudomonadaceae	Pseudomonas	Sp.	211	0	0			
98	Pseudomonadaceae	Pseudomonas	Sp.	225	0	0			
104	Pseudomonadaceae	Pseudomonas	Sp.	272	0	0			
11	Rhizobiaceae	Rhizobium	Sp.				131	1	0
34	Sphingomonadaceae	Sphingomonas	Sp.				9	2	0
36	Rhodospirillaceae	Azospirillum	amazonense				130 150 198	1 1 1	0 0 0
82	Staphulococcaceae	Staphulococcus	Sp.	218	0	0			
12	Xanthomonadaceae	Stenotrophomonas	Sp.	50	0	0	29 34	1 1	2 2
18	Xanthomonadaceae	Stenotrophomonas	maltophilia	107	0	0			
58	Xanthomonadaceae	Stenotrophomonas	maltophilia	84 262	0 0	0 0	7	0	0
63	Xanthomonadaceae	Luteibacter	rhizovicina				14	0	0
70	Xanthomonadaceae	Stenotrophomonas	maltophilia	93 173 267	0 0 0	0 0 0			
87	Xanthomonadaceae	Stenotrophomonas	maltophilia	94	0	0			
102	Xanthomonadaceae	Xanthomonas	Sp.	75	0	0			

Contig (1)	Family (2)	Genus (3)	Species (4)	Alluvial soil			Acid soil		
				Identify (5)	Cellulase (6)	Chitinase (7)	Identify (8)	Cellulase (9)	Chitinase (10)
9	Ceratobasidiaceae	Ceratobasidium	Sp.				23	0	0
6	Hypocreaceae	Hypocrea	lixii	27 28	1 1	1 2			
3	Mucoraceae	Mucor	hiemalis	24 25 29 30 31 33 34 35 36	0 1 0 0 1 0 0 0 0	1 2 1 1 1 1 1 1 1			
1	Nectriaceae	Nectria	mauritiicola				4 5 6 7 9 10 17 19 44	1 1 1 2 1 1 2 1 1	1 1 2 2 1 1 2 1 1
2	Nectriaceae	Gibberella	sacchari	2 22 42 46 47	1 1 2 1 1	1 1 0 0 0	12	1	1

Appendix 2: Summary of fungal contigs identified in the rice stubble from two fields.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
7	Pleosporaceae	Dendryphiella	Sp.				39 40 41	1 1 1	0 0 0
4	Sordariaceae	Neurospora	tetrasperma	26 32 37 43	1 0 0 0	0 2 0 0			
11	Sporormiaceae	Preussia	minimoides				8 11 13 14 16 18 20	2 2 3 1 3 2 2	1 2 1 2 0 0 1
10	Trichocomaceae	Aspergillus	flavus	3	1	0			
5		Fusarium	chlamydosporum	1	1	1			
8		Stachybotrys	bisbyi				38	1	2
12		Corynespora	cassiicola				15	2	0

Appendix 3: The effect of fungi on rice radicle length

	Isolate	No. with shoot	colony	Radicle length (cm) G1	G2	G3	G3/G1
Beneficial effect							
Nectria mauritiicola	5	24,5	4	1,5	11	11,5	7,7
Nectria mauritiicola	9	24	7	3	8,5	13	4,3
Preussia minimoides	13	23,5	24	5,5	7,5	10,5	1,9
Nectria mauritiicola	4	22,5	9,5	4,5	9,5	8,5	1,9
Corynespora cassiicola	15	20,5	25	4	9	7,5	1,9
Nectria mauritiicola	19	22,5	5,5	3,5	12,5	6,5	1,9
Nectria mauritiicola	6	23	3	4	12,5	6	1,5
Dendryphiella sp.	39	21,5	10,5	4	11,5	6	1,5
Nectria mauritiicola	44	19,5	8,5	3,5	12	4	1,1
Nectria mauritiicola	10	23	7,5	4,5	8,5	5	1,1
Preussia minimoides	14	22,5	5	6	10,5	6,5	1,1
Preussia minimoides	18	22	25	6,5	8,5	7	1,1
Nectria mauritiicola	17	22,5	5,5	6	10,5	6	1,0
Nectria mauritiicola	7	21	9	5,5	12	4	0,7
Preussia minimoides	20	22	25	5,5	13,5	3	0,5
Preussia minimoides	8	23	25	5	15,5	2,5	0,5
Preussia minimoides	16	23	25	6,5	13,5	3	0,5
Gibberella sacchari	12	21,5	15,5	11,5	5,5	4,5	0,4
Control		**20,5**	**2**	**6**	**15**	**2**	**0,3**
Deleterious effect							
Preussia minimoides	11	22,5	25	6,5	13,5	2	0,3

With: G1: 0.1- 3.9 cm; G2: 4-4.9 cm; G3: 6-7 cm

Appendix 4: The effect of bacteria on rice radicle length

Species	Isolate	No. with shoot	Colony	Radicle length (cm)			
				G1	G2	G3	G3/G1
Benefical effect							
Bacillus sp.	120	21,5	4,5	3	5,5	13,5	4,5
Azospirillum	150	20	7,5	2	10	9	4,5
Unidentified bacterium	279	23	5,5	4	4	15	3,8
Stenotrophomonas sp.	34	23	6,5	3,5	7	12,5	3,6
Citrobacter sp.	39	21	7	3,5	6	12,5	3,6
Bacillus cereus	249	21,5	5,5	3,5	6	12	3,4
Pantoea dispersa	235	21	4,5	4	3,5	13,5	3,4
Pantoea dispersa	242	21,5	11	3	9,5	10	3,3
Control	**1**	**22**	**4,5**	**3**	**9,5**	**9,5**	**3,2**
Deleterious effect							
Unidentified bacterium	149	22,5	4,5	3,5	8,5	10,5	3,0
Bacillus subtilis	158	22,5	4,5	3	11,5	8,5	2,8
Enterobacter sp.	65	21,5	6	4	7	10,5	2,6
Sphingomonas	9	20,5	7,5	3,5	9	9	2,6
Flexibacter Flexibacter	147	22	6,5	3	11,5	7,5	2,5
Pantoea dispersa	236	23	4	5	6	12	2,4
Microbacterium laevaniformas	129	23,5	7,5	5	6,5	12	2,4
Stenotrophomonas sp.	29	22,5	5,5	4	5,5	9,5	2,4
Paenibacillus	1	22,5	7	5,5	4,5	12,5	2,3
Herbaspirillum sp.	135	22,5	6,5	4,5	10	8	1,8
Uncultured Escherichia	32	23,5	7,5	7	4,5	12	1,7
Bacillus pumilus	90	19,5	9,5	4,5	8	7	1,6
Chitinophaga sp.	197	19	7,5	5	7,5	7	1,4
Burkholderia gladioli	68	23,5	5,5	7	7	9,5	1,4
Pantoea dispersa	243	18	11	5,5	11	6	1,1
Flexibacter sancti	22	23,5	4,5	7	9,5	7	1,0
Unidentified bacterium	31	19,5	10	6	10	5,5	0,9
Serratia sp.	30	16	8	9	4	3,5	0,4
Burkholderia gladioli	142	23	5	13	9,5	0,5	0,0
Pantoea dispersa	181	18,5	8,5	16,5	2	0	0,0
Burkholderia gladioli	33	13	6,5	8	0	0	0,0
Bacillus pumilus	146	7,5	6	2	0	0	0,0

Appendix 5: Bacterial functional groups two different sites

Bacteria-VN	**Count**	**Pi**	**Log(Pi)**	**Pi * Log(Pi)**
Cellulase	61	0.42	-0.86	0.36
Cellulase+ Chitinase	2	0.01	-4.28	0.06
Neutral	81	0.56	-0.58	0.32
Total	144			**H´= 0.74**
Bacteria-HA				
Cellulase	44	0.38	-0.96	0.38
Cellulase+ Chitinase	18	0.16	-1.86	0.16
Neutral	53	0.46	-0.77	0.46
Total	115			**H´= 1.02**

Appendix 6: Fungal functional groups in two different rice fields

Fungi-VN	**Count**	**Pi**	**Log(Pi)**	**Pi * Log(Pi)**
Cellulase	5	0.23	-1.48	0.23
Chitinase	8	0.36	-1.01	0.36
Cellulase+ Chitinase	7	0.32	-1.15	0.32
Neutral	2	0.09	-2.40	0.09
Total	22			**H´**= 1.29
Fungi-HA				
Cellulase	6	0.26	1.34	0.26
Cellulase+ Chitinase	16	0.70	0.36	0.70
Neutral	1	0.04	3.14	0.04
Total	23			**H´= 0.74**

Appendix 7: Shannon Weaver index of bacterial and fungal families in two sites

Hoa An	Count	P_i	$Log(P_i)$	$P_i * Log(P_i)$
Bacillaceae	14	0.12	-2.11	0.26
Burkholderiaceae	34	0.30	-1.22	0.36
Caulobacteraceae	1	0.009	-4.75	0.04
Enterobacteriaceae	26	0.23	-1.49	0.34
Flavobacteraceae	1	0.009	-4.75	0.04
Flexibacteraceae	4	0.04	-3.36	0.12
Microbacteriaceae	9	0.08	-2.55	0.20
Moraxellaceae	1	0.009	-4.75	0.04
Neisseriaceae	4	0.04	-3.36	0.12
Oxalobacteraceae	6	0.05	-2.95	0.15
Paenibacillace	1	0.009	-4.75	0.04
Pseudomonadaceae	5	0.04	-3.14	0.14
Rhizobiaceae	1	0.009	-4.75	0.04
Rhodospirillaceae	3	0.03	-3.65	0.10
Sphingomonadaceae	1	0.009	-4.75	0.04
Xanthomonadaceae	4	0.04	-3.36	0.12
Total	115			H′= 2.14
Vinh Nguon				
Bacillaceae	41	0.29	-1.26	0.36
Burkholderiaceae	21	0.15	-1.93	0.28
Enterobacteriaceae	24	0.17	-1.79	0.30
Microbacteriaceae	3	0.02	-3.87	0.08
Moraxellaceae	4	0.03	-3.58	0.10
Pseudomonadaceae	41	0.29	-1.25	0.36
Staphylococcaceae	1	0.007	-4.97	0.034
Xanthomonadaceae	9	0.06	-2.77	0.17
Species Count: 8	144			H′=1.68

Appendix 8: Fungal families identified in rice stubble from two soils

Fungal family	**Count**	**Pi**	Ln (Pi)	Pi * Ln (Pi)
Vinh Nguon				
Hypocreaceae	2	0.09	-2.40	0.22
Mucoraceae	9	0.41	-0.89	0.37
Nectriaceae	5	0.23	-1.48	0.34
Sordariaceae	4	0.18	-1.7	0.31
Trichocomaceae	1	0.05	-3.09	0.14
Uncultured fungi	1	0.05	-3.09	0.14
Species Count: 6	**Total Count:** 22			**H´=1.51**
Hoa An				
Ceratobasidiaceae	1	0.04	-3.14	0.14
Nectriaceae	10	0.43	-0.83	0.36
Pleosporaceae	3	0.13	-2.04	0.27
Sporormiaceae	7	0.30	-1.19	0.36
Uncultured fungi	2	0.09	-2.44	0.21
Species Count: 5	**Total Count:** 23			**H´=1.34**

Table 9: The experimental design of rice straw decomposition with the combinations of different species and functional groups

Function group	Genus	Isolate	Contig	1 sp	2 sp 1 fg	2 sp 2fg	6 sp 6 fg	16 sp[1] 6 fg[2]
High cellulolytic fungi	*Gibberella*	42	2	1	1,3	1,11	1,4,9,11,15, 14	1,2,3,4,5,6,7,8,9,10,1115,16 12,13,14
	Dothidales	18	11	2	4,5	6, 15	3,5,8,10,16, 13	
	Sarocladium	7	1	3	7,9	8, 2	2,6,7,10,16, 14	
Low cellulolytic fungi	*Dendryphiella*	40	7	4	10,11	16,3	2,5,9,11,15, 12	
	Rhiomucor	25	3	5	12,13	11, 7		**(1): Number of species**
	Stachybotrys	38	8	6	15,16	6, 10		**(2): Number of functional group**
High cellulolytic bacteria	*B. subtilis*	162	10	7		7, 16		http://www.mathsyear2000.org/explor
	B. pumilus	232	25	8		1,4		er/
	Pantoea	181	14	9		11,16		randomiser/#integers
High cellulolytic + high chitinolytic acteria	*Flexibacter*	179	43	10		5,8		
	Burkholderia	68	16	11		2,14		
Low cellulolytic + high chitinolytic bacteria	*B. pumilus*	146	25	12		13,8		
	Serratia	30	4	13		12,4		
	Serratia	154	4	14				
N fixing bacteria	*Rhizobium*	131	11	15				
	Herbaspirillum	156	15	16				
	Control							