Introduction of crop rotation and rice straw application in a former flooded rice system and their impact on the microbial community in bulk soil and the rhizosphere of *Zea mays*

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Sarah Athea Maarastawi

aus

Bonn

Bonn, August 2018

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn.

1. Gutachterin: Prof. Dr. Claudia Knief
2. Gutachter: Prof. Dr. André Lipski

Tag der Promotion: 20.11.2018

Erscheinungsjahr: 2018

Die Natur hat zu jederzeit Recht, und das gerade am gründlichsten, wo wir sie am wenigsten begreifen. –Johann Wolfgang von Goethe

Teile der in dieser Dissertation beschriebenen Ergebnisse sind in folgenden Originalpublikationen veröffentlicht:

Frindte, K., Maarastawi, S. A., Lipski, A., Hamacher, J., and Knief, C. (2017). Characterization of the first rice paddy cluster I isolate, *Methyloterricola oryzae* gen. nov., sp. nov. and amended description of *Methylomagnum ishizawai*. *Int. J. Syst. Evol. Microbiol.* 67, 4507–4514. doi:10.1099/ijsem.0.002319.

Maarastawi, S. A., Frindte, K., Linnartz, M., and Knief, C. (2018). Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of *Zea mays*. *Front. Microbiol.* doi:10.3389/fmicb.2018.01295.

Maarastawi, S. A., Frindte, K., Geer, R., Kröber, E. and Knief, C (2018). Temporal dynamic and compartments specific rice straw degradation in bulk soil and the rhizosphere of maize. *Soil Biology and Biochemistry*. Doi.org/10.1016/j.soilbio.2018.09.028

Weitere Teile werden in folgenden Publikation veröffentlicht:

Maarastawi, S. A., Frindte, K., Bodelier, P. and Knief, C. Rice straw serves as additional carbon source for rhizosphere microorganisms (planned submission to Soil Biology and Biochemistry)

Table of contents

List of abbreviations

AMS	ammonium mineral salts
ANOSIM	analysis of similarity
ANOVA	analysis of variance
DAPI	4',6-diamidino-2-phenylindole
DGGE	denaturing gradient gel electrophoresis
EF	enrichment factor
FAME	fatty acids methyl ester
FOM	fresh organic matter
GC-FID	Gas chromatography coupled to flame ionization detector
GC-IRMS	Gas chromatography coupled to stable isotope ratio mass spectrometry
GC-MS	Gas chromatography coupled to mass spectrometry
ICON	Introduction of non-flooded crops in rice-dominated landscapes and its impact on carbon, nitrogen and water cycles
IRRI	International Rice Research Institute
MM	maize monoculture
MOBs	methanotrophic bacteria
Mha	million hectares
MR	rice-maize crop rotation
NMDS	non-metric multidimensional scaling
NMS	nitrate mineral salts
OTU	operational taxonomic unit
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	priming effect
PHB	poly-β-hydroxybutyrate
PLFA	phospholipid fatty acid
p.p.m.	part per million
QS	quorum sensing
RPC1	rice paddy cluster I
RR	rice monoculture
RuBisCO	ribulose-1,5-bisphosphat-carboxylase
RuMP	ribulose monophosphate
SIP	stable isotope probing
SOM	soil organic matter

Summary

Rice is one of the most important staple foods with an increasing demand, because of the rising world population. To reach the highest possible yield, rice is mostly cultivated under flooded conditions. The constant flooding of rice fields results in anoxic conditions in soil, providing an optimal habitat for anaerobic microorganisms. The occurrence of methane (CH_4) producing *Archaea* leads to high methane emissions during rice cultivation. The produced methane is the main substrate for methanotrophic bacteria that oxidize it to carbon dioxide (CO_2) and therefore represent a methane sink within rice fields. Nevertheless, the traditional cultivation of rice accounts for about 10% of the anthropogenic sources of methane emissions and requires high amounts of water for the irrigation of the fields.

To decrease water consumption and minimize methane emissions, an altered crop rotation by introducing plants such as maize, cultivated under upland conditions, is considered. Rice farmers in the Philippines increasingly introduce maize as crop in the dry season, followed by rice in the wet season. However, previous studies observed that such a crop rotation promotes the development of desiccation cracks and due to that a leaching of carbon (C) and nitrogen (N), whereby an increased emission of nitrous oxide (N_2O) is also observed. To prevent these losses, rice straw can be applied as mulch to conserve the soil moisture content and stabilize soil aggregates.

The introduction of crop rotation and the straw application may lead to changes in the microbial community structure. Such changes can be induced by changes in the cultivation technique (from flooded to non-flooded conditions), by introduction of maize as crop in a former rice system as well as by provision of fresh organic matter in form of rice straw. How the microbial community responds to the introduction of crop rotation and straw application is a central question in this thesis. Furthermore, the analysis of the microbial community will indicate whether a combination of these two agricultural management practises reduces the problem of high methane emissions. Therefore, it is important to investigate the changes in microbial community structure in the dry season during maize cultivation.

I analysed the microbial communities in rice field soils in comparison to rice-maize crop rotation soils. The microbial community of paddy soils is well studied and characterized by the presence of methanogens and methanotrophs. I additionally focussed on the rice phyllosphere and isolated methanotrophs that are potentially able to consume methane emitted by rice plants. To have a closer look at the impact of crop rotational changes on bacteria, archaea and fungi, I analysed the microbial communities during maize cultivation in rice monoculture soils, rice-maize crop rotation soils and, as a control, in a maize monoculture soils, via amplicon

sequencing. This revealed that microbial communities in soils under crop rotation have a higher similarity to those in rice soils than in maize soils. However, differences between the communities in rice soils compared to crop rotational soils were mainly due to a depletion of anaerobic microbes in crop rotation soils.

The impact of straw addition on the microbiota in the different cultivated soils was superimposed by other factors like crop rotation, compartment (bulk soil versus rhizosphere) and time. Therefore, I investigated the straw degrading community in more detail in a soil under crop rotation by applying highly labelled ^{13}C-rice straw to the bulk soil and rhizosphere of maize and investigated the active straw-degrading labelled bacterial and fungal community with stable isotope probing (SIP) and further amplicon sequencing. The results showed that straw degradation was performed by aerobic microorganisms in soils under crop rotation and underwent a clear temporal succession. In the initial phase, mainly fast-growing bacteria became labelled and consumed readily degradable carbon compounds, followed by a labelling of mainly fungi that degrade more complex carbon compounds. In the rhizosphere I detected partly different microorganisms as labelled than in bulk soil, indicating that host plant specific taxa benefit from straw in the rhizosphere. Nevertheless, the lower label intensity in the rhizosphere indicates that rhizosphere organisms use straw as additional carbon source with lower efficiency besides using rhizodeposits. To investigate specifically the root exudate consuming microorganisms in the rhizosphere, I conducted a labelling experiment of maize with $^{13}CO_2$ and subsequent phospholipid fatty acid (PLFA)-SIP analysis. The results confirmed that the addition of straw impacts the uptake of root exudates. Obviously, a simultaneous use of root exudates and straw takes place, because straw addition resulted in a decreased uptake of root exudates.

This thesis provides evidence that the introduction of crop rotation leads to an altered microbial community in the bulk soil and rhizosphere of maize, which remains more similar to the community in paddy fields than in upland maize fields. Nevertheless, the investigated crop rotational soils were characterized by a depletion of anaerobic bacteria and methanogenic archaea in comparison to rice soils. The additional straw application leads only to a stimulation of aerobic microbes in the rhizosphere of maize as well as in bulk soil, whereas the straw application in paddy soils leads to a significant increase of methanogens in both compartments, as known from literature. The depletion of methanogens leads to the assumption that a crop rotation of rice, followed by maize, and straw addition during the dry season is a promising strategy to reduce methane emissions. Furthermore, it is known that the introduction of an upland crop in a paddy system reduces water consumption and that straw addition simultaneously conserves moisture and nutrients in soil.

Zusammenfassung

Reis ist eines der Hauptnahrungsmittel weltweit, dessen Nachfrage aufgrund der wachsenden Weltbevölkerung immer weiter steigt. Um den höchstmöglichen Ertrag zu erzielen und somit den globalen Bedarf zu decken, wird Reis überwiegend unter gefluteten Bedingungen angebaut. Durch die kontinuierliche Flutung der Felder entstehen anoxische Bedingungen im Reisboden, die ein optimales Habitat für anaerobe Mikroorganismen bieten. Das Vorkommen von anaeroben Methan-produzierenden *Archaea* führt zu einer hohen Methanemission während der Reiskultivierung. Das produzierte Methan (CH_4) ist das Hauptsubstrat für methanotrophe Bakterien, die dieses zu Kohlenstoffdioxid (CO_2) oxidieren und somit die einzige Methansenke innerhalb der Reisfelder darstellen. Trotz dieser Methansenke macht der traditionelle Reisanbau ein Zehntel der anthropogen verursachten Methanemissionen aus. Außerdem werden zur Flutung der Felder beim Reisanbau hohe Mengen Wasser verbraucht.

Eine Methode um den Wasserverbrauch und die Methanemission zu senken ist die Einführung eines Fruchtfolgewechsels mit Pflanzen, deren Kultivierung keiner Flutung bedarf. Auf den Philippinen wurde deswegen ein Fruchtfolgewechsel eingeführt zwischen Reisanbau in der Regenzeit und Maisanbau in der Trockenzeit. Studien, die sich mit der Untersuchung dieser Fruchtfolgewechselböden beschäftigten, belegten, dass dieses Kultivierungsverfahren zu der Entstehung von Trockenrissen im Boden führt. Durch die Trockenrisse kommt es wiederum zu einer vermehrten Auswaschung von Kohlenstoff (C) und Stickstoff (N) im Boden und zu einer erhöhten Emission von Distickstoffoxid (N_2O). Um diese Verluste zu kompensieren, wird Reisstroh in den Boden inkorporiert und als Mulch verwendet. Dies verringert die Evaporation, hilft dadurch dabei die Feuchtigkeit im Boden zu halten und die Bildung von Trockenrissen zu verringern.

Die Einführung von einem Fruchtfolgewechsel als auch die Strohapplikation kann zu einer Veränderung der mikrobiellen Gemeinschaft im Boden führen. Diese Veränderung der Gemeinschaft kann sowohl durch die Veränderungen der Bewirtschaftung (von gefluteten auf nicht gefluteten Bedingungen), durch die Einführung von Maispflanzen als auch durch das Bereitstellen von frischem organischem Material in Form von Stroh hervorgerufen werden. In wie weit sich die mikrobielle Gemeinschaft durch die Einführung von Fruchtfolgewechsel und Strohapplikation verändert ist eine zentrale Fragestellung in dieser Arbeit. Außerdem kann die Analyse der mikrobiellen Gemeinschaft einen Hinweis darauf geben ob eine Kombination von Fruchtfolgewechsel und Strohapplikation die Methanemission von Reisfeldern senken kann. Demzufolge ist es von Bedeutung, die mikrobielle Gemeinschaft in der Trockenzeit während der Kultivierung von Mais zu untersuchen.

Um dies zu ermitteln wurde die mikrobielle Gemeinschaft in Reisfeldern im Vergleich zu Feldern, die einen Fruchtfolgewechsel unterlagen, analysiert. Die mikrobielle Gemeinschaft von gefluteten Reisböden ist charakterisiert durch die Präsenz von methanogenen Archaeen und methanotrophen Bakterien. In dieser Arbeit wurde ebenfalls die Reisphyllosphäre näher betrachtet, wodurch Methanotrophe isoliert und beschrieben werden konnten, die potentiell in der Lage sind, das von der Reispflanze emittierte Methan zu konsumieren. Um den Einfluss von einem Fruchtfolgewechsel zwischen Reis und Mais auf die Bakterien, Archaeen und Pilze näher zu betrachten, wurde die mikrobielle Gemeinschaft während der Kultivierung von Mais in Reis- und Maismonokulturböden sowie in Fruchtfolgewechselböden mittels Amplicon Sequenzierung, analysiert. Die Ergebnisse zeigten, dass die mikrobielle Gemeinschaft in Fruchtfolgewechselböden mehr der Gemeinschaft in Reismonokultur Böden ähnelt als der in Maismonokultur Böden. Ein Unterschied zwischen der Gemeinschaft in Fruchtfolgewechselböden und Reisböden resultierte jedoch vor allem durch eine Reduzierung von anaeroben Mikroorganismen in Fruchtfolgewechselböden.

Der Einfluss von Stroh auf die Mikrobiota war von anderen Faktoren wie Fruchtfolgewechsel, Bodenkompartiment (nicht durchwurzelter Boden *versus* Rhizosphäre) und Inkubationszeit überlagert. Um einen detaillierteren Einblick in die stroh-abbauende Mikrobiota zu erhalten, applizierte ich ^{13}C-markiertes Reisstroh in einen Fruchtfolgewechselboden. Die markierte mikrobielle Gemeinschaft im nicht durchwurzelten Boden sowie in der Rhizosphäre von Mais wurde anschließend mit Hilfe der Stabilen Isotopen Beprobung (SIP) auf DNA Ebene und anschließender Amplicon Sequenzierung untersucht. Die Analysen zeigten, dass die Strohdegradation in dem Fruchtfolgewechselboden von aeroben Mikroorganismen durchgeführt wird. Außerdem konnte eine klare zeitliche Sukzession des Strohabbaus beobachtet werden. Der initiale Strohabbau erfolgte hauptsächlich durch schnell wachsende Bakterien, die einfach verfügbare Kohlenstoffverbindungen des Strohs konsumieren können. Anschließend degradierten hauptsächlich Pilze die schwerer verfügbaren Kohlenstoffverbindungen. In der Rhizosphäre waren zum Teil andere Taxa am Strohabbau beteiligt als im nicht durchwurzelten Boden. Dies weist darauf hin, dass in der Rhizosphäre wirtspflanzenspezifische Mikroben vom Stroh profitieren. Die geringere Markierungsintensität in der Rhizosphäre deutet darauf hin, dass diese Mikroorganismen das Stroh als zusätzliche Kohlenstoffquelle zur Rhizodeposition nutzen, allerdings mit geringerer Effizienz als im nicht durchwurzelten Boden. Um spezifisch die Wurzelexsudat konsumierende mikrobielle Gemeinschaft in der Rhizosphäre von Mais zu untersuchen, wurden die Maispflanzen mit $^{13}CO_2$ markiert und anschließend eine Phospholipid-Fettsäure (PLFA)-SIP durchgeführt. Die Ergebnisse bestätigten, dass die Applikation von Stroh die Aufnahme von Wurzelexsudaten

beeinflusst. Dies weist darauf hin, dass eine gleichzeitige Nutzung von Wurzelexsudaten und Stroh stattfindet, da durch die Strohapplikation ein verringerter Konsum von Wurzelexsudaten beobachtet werden konnte.

Diese Arbeit zeigt, dass die mikrobielle Gemeinschaft von Böden, die einem Fruchtfolgewechsel unterliegen, mehr der mikrobiellen Gemeinschaft von Reisböden ähnelt als der Gemeinschaft von Maisböden. Allerdings sind die Fruchtfolgewechselböden im Vergleich zu Reisböden gekennzeichnet durch eine Reduktion Reisfeld-typischer anaerober Mikroorganismen und demzufolge auch durch eine Reduktion von Methanogenen. Dieser Trend konnte sowohl in der Rhizosphäre von Mais als auch im nicht durchwurzelten Boden beobachtet werden. Die zusätzliche Strohapplikation in diesen Böden führt zu einer Stimulation von aeroben Mikroorganismen sowohl in der Rhizosphäre von Mais als auch im nicht durchwurzelten Boden. Im Unterschied dazu geht aus der Literatur hervor, dass die Zugabe von Stroh in Reismonokulturen zu einem signifikanten Zuwachs an Methanogenen sowohl in der Rhizosphäre von Reis als auch im nicht durchwurzelten Boden führt. Demzufolge könnte die Kombination von Fruchtfolgewechsel und Strohapplikation in Reisböden eine vielversprechende Strategie zur Reduktion der Methanemission sein. Außerdem ist bereits bekannt, dass der Wasserverbrauch durch die Einführung von Mais reduziert wird und die Strohapplikation gleichzeitig die Feuchtigkeit im Boden konserviert sowie dazu führt, dass dem Boden Nährstoffe zurückgeführt werden.

1. Introduction

1.1. Cultivation of rice and maize

Rice and maize are the most important staple foods worldwide. Together with wheat they account for approximately 85 % of the world cereal exports (Parry et al., 2004). Because of that, the used agricultural area for the cultivation of these crops is immense. Especially in Asia these crops contribute highly to food security and income. The crop area of rice and maize in Asia makes up an area of about 64 million hectares (Mha) (55 Mha rice and 9 Mha maize) (Timsina et al., 2010). The upland agricultural system in Asia is already dominated by maize cultivation, not only for the food industry but also because of the increasing demand of maize for poultry fattening and biofuel production (Rosegrant, 2008; Weller et al., 2016).

Nevertheless, the production of rice is still much higher in Asia and feeds more people than any other crop (Wassmann et al., 2009). In fact more than 3 billion people consume more than 100 kg rice per person and year (Van Nguyen and Ferrero, 2006). Ninety percent of the worldwide rice is produced and consumed in Asia and most of it is cultivated under flooded conditions (Fuhrmann et al., 2018; Wassmann et al., 2009). Rice production under flooded conditions prevents land subsidence, leads to a lower soil erosion and a higher soil organic matter content in comparison with other production systems (Bouman et al., 2007). However, the environmental problems, which occur due to the traditional rice cultivation, are greater than its benefits. In comparison with other crops, rice requires two to three times more water per hectare, as the water consumption adds up to 3000 – 5000 l/kg rice. Especially in Asia, farmers need 50% of freshwater to irrigate paddy fields (Tuong et al., 2005). Because of the scarcity of global water resources for agriculture, the possibility to expand the area for further paddy systems will remain very limited (Van Nguyen and Ferrero, 2006). Further, rice cultivation comprises significant methane emissions, accounting for about 10% of all anthropogenic methane emission (Nazaries et al., 2013). Methane contributes with up to 20 – 30 % to the global warming effect and its atmospheric concentration increased from 0.7 p.p.m. (part per million) until the beginning of the 19th century to 1.778 p.p.m. in 2005 (Forster et al., 2007). It is the second most significant greenhouse gas after CO_2 and is 25 times more potent than CO_2 as greenhouse gas (Forster et al., 2007; Nazaries et al., 2013).

1.2. Microbial habitats in rice and maize fields

Methane is produced in the anoxic parts of paddy soils by methanogenic archaea as an end product of the anaerobic degradation of organic matter (Breidenbach and Conrad, 2015). Methanogens rely on the presence of a larger bacterial consortium including hydrolytic, fermenting, syntrophic and acetogenic bacteria, which constitute the prior steps of degradation of organic matter (Nazaries et al., 2013). The methane producing archaea can be classified into acetotrophic methanogens, which dismutate acetate to CH_4 and CO_2 and hydrogenotrophic methanogens, reducing CO_2 with H_2 to CH_4 (Conrad, 2007).

Methanogens inhabit the anoxic bulk soil (figure 1.1) of rice fields, whereas methanotrophic bacteria can be found in the oxic zones like in the oxic surface soil, in the rhizosphere as well as in the phyllosphere (figure 1.1) (Knief et al., 2012). Methanotrophs play an important role in attenuating the methane emission of rice field soils by up to 40 % (Conrad, 2009; Lüke et al., 2010). The most important CH_4 sink is the root-associated methane oxidation. This oxic-anoxic boundary develops due to oxygen (O_2) diffusion from the atmosphere through the aerenchyma of the rice plant, resulting in a release of O_2 into the soil around the rice roots and therefore supports the methane oxidation (Lüke et al., 2010). Methane produced in the anoxic bulk soil can also be emitted passively by plants through the aerenchyma, serving as transport way for methane to the atmosphere (Iguchi et al., 2012). Hence, the rice phyllosphere can also serve as habitat for methanotrophs (Knief et al., 2012). In rice soil and the rice rhizosphere genera like *Methylocystis*, *Methylosinus*, *Methylomonas*, *Methylosarcina*, *Methylobacter* or *Methylosoma* were found frequently by cultivation-independent methods (Conrad, 2007; Knief et al., 2012). Nevertheless, the isolation of methanotrophs from rice fields remains more challenging. The number of methanotrophs isolated from rice fields is rather low, mostly the common genera *Methylocystis*, *Methylosinus* and *Methylomonas* have been isolated from rice fields (Heyer et al., 2002; Ogiso et al., 2012). Especially in the rice phyllosphere the detection of methanotrophs with cultivation independent methods was possible, but the cultivation of those taxa remains challenging so far. In general, the majority of the methanotrophs detected in environmental samples was derived from cultivation-independent studies. Most of the cultured methanotrophs belong to the well-known genera *Methylocystis*, *Methylosinus*, *Methylomonas*, *Methylobacter*, *Methylocaldum* or *Methylomicrobium*. This means that only a small fraction of methanotrophic diversity is represented by cultivated strains (Knief, 2015).

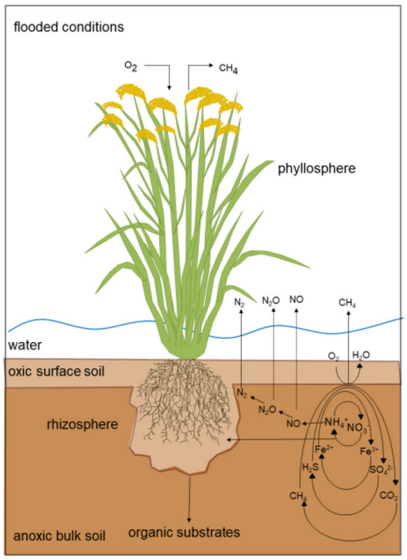


Figure 1.1: Scheme of a rice field, illustrating the major habitats of methanogenic (anoxic bulk soil) and methanotrophic (oxic surface soil, rhizosphere, phyllosphere) microorganisms. Additionally, the redox cycling and methane emission pathways are shown. The diagram has been modified from Conrad (2007).

Oxygen is one of the most important factors shaping habitats and therefore influencing the microbial community due to prevalent redox conditions. Whereas paddy fields consist of large anoxic parts so that the degradation of organic matter leads to a production of methane, upland fields as used for maize cultivation are well aerated. Due to the high redox potential, methane production and emissions in upland maize soils were rarely reported (Weller et al., 2015), and can only occur in small anoxic micro-niches to a generally small extent (Megonigal and Guenther, 2008).

Maize fields are characterized by a higher production and emission of N_2O than rice fields. The water-filled pore space of drained soils is about 50 – 70 %, and the maximum of N_2O emissions was determined to be within this range (Davidson et al., 2000). The N_2O production in upland soils is mainly related to rainfall or irrigation events as well as to increased fertilisation as a result of tight coupling of nitrification and denitrification processes by microbes (Snyder et al., 2009; Weller et al., 2016). Due to fertilisation, ammonia, the substrate for nitrification, is available at high concentrations and high amounts of nitrate (NO^{3-}) can be produced. Nitrate, again, can be used for denitrification in anoxic micro-niches. During this process, a variable portion of N is often emitted as N_2O gas (Snyder et al., 2009). So, flushes of N_2O can occur when previously well-aerated soils become moistened or saturated from precipitation or irrigation (Snyder et al., 2009). In contrast, the N_2O emission from paddy fields are mostly negligible, because the anaerobic conditions in flooded rice systems favour complete denitrification to nitrogen gas (N_2), and the necessary substrate nitrate cannot be regenerated under anoxic conditions (Fan et al., 2014; Weller et al., 2015, 2016).

1.3. Introduction of crop rotation in a paddy system

The traditional rice cultivation causes major environmental problems as described before. The increasing demand of water in municipal and industrial sectors and the climatic changes necessitate revision of rice production systems considering the high water consumption and methane emissions. In the past, several management strategies have been developed to preserve water and to minimize methane emissions during rice cultivation, e.g. additional drainage periods in the fields, early timing of midseason drainage or intermittent drainage of the fields (Wassmann et al., 2000b, 2000a; Yagi et al., 1996)

However, in consideration of the simultaneously increasing demand of maize for poultry fattening and biofuel production, farmers in subtropical and tropical Asia diversify their cropping systems by introducing a crop rotation system with rice in the wet season and maize as upland crop in the dry season (Weller et al., 2015). The seasonal changes between dry and wet conditions in the soil lead to the formation of desiccation cracks during maize cultivation, which causes the loss of water, dissolved organic carbon, and an additional risk of nitrogen losses due to the increased release of the greenhouse gas N_2O (Fuhrmann et al., 2018; He et al., 2015; Weller et al., 2015).

N_2O can be produced by at least three processes: i) during nitrification as by-product of the oxidation of hydroxylamine (NH_2OH) to nitrite (NO^{2-}), ii), during nitrifier denitrification, which is the reduction of NO_2^- by ammonium oxidizing microbes under oxygen-limited conditions and iii) during denitrification under anoxic conditions by the reduction of nitrate, which is produced from nitrification or applied as fertiliser (Kool et al., 2011; Wu et al., 2018; Zhang et al., 2016). During maize cultivation in a rice-maize crop rotation soil, the conditions for all three processes are provided. Nitrification can take place in the aerated parts and nitrifier denitrification or denitrification can occur in anoxic water saturated micro-niches during maize cultivation or during rice cultivation in the anoxic bulk soil. The alternating moist-dry soil conditions after irrigation or heavy rainfall events stimulate the nitrification-denitrification processes and thus promote N_2O production and emissions in a rice-maize crop rotation system (Breidenbach et al., 2015).

The variances of cultivation under flooded conditions vs. non-flooded conditions will change the redox conditions in soils, because innate rice fields are exposed to long periods of drainage along with upland field conditions (Breidenbach et al., 2015). Aeration of soil during maize cultivation can cause oxygen stress for anaerobic microorganisms and enhance the activity of aerobic microorganisms at the same time (Breidenbach et al., 2015). Thus, the periodical

changes in oxygen availability in these soils are linked to a substantially different microbial carbon conversion process under the different conditions. In aerobic soils the mineralisation of organic matter leads to a production of CO_2 due to the oxidation of carbon compounds by diverse microorganisms including bacteria as well as fungi (Kimura et al., 2004). In previous studies a significant increase of CO_2 producing methanotrophs and a decrease of methanogens in drained rice soils was observed (Ma et al., 2013; Ma and Lu, 2011). Methane production in drained rice soils can only occur in anoxic micro-niches by methanogens and is generally of small extent (Megonigal and Guenther, 2008). Under anoxic conditions during rice cultivation, the degradation of organic matter is more complex (Liesack et al., 2000). After flooding, a cascade of alternative electron acceptors following the redox potential can be used instead of oxygen (nitrate, ferric iron, sulphate; figure 1.1). However, these oxidants are usually depleted after several days (Breidenbach et al., 2015). Nevertheless, these electron acceptors can be partly regenerated at oxic-anoxic interfaces (surface soil, rhizosphere) (Conrad and Frenzel, 2003). After the complete depletion of these electron acceptors, especially in deeper soil layers, methanogenesis is initiated by reducing CO_2 as a last step of the anoxic degradation of organic matter (Liesack et al., 2000).

Soils undergoing crop rotation between upland-upland plants (like wheat-soybean) as well as between wetland-upland plants (like rice-mungbean or maize) harbour a significantly different bacterial community composition, diversity and abundance compared to monoculture soils (Lopes et al., 2014; Xuan et al., 2012; Yin et al., 2010). Especially in rice-maize crop rotation soils the archaeal community composition changed dramatically, characterized by a decrease of anaerobic methanogens and an increase of aerobic archaea (Breidenbach et al., 2015; Ma et al., 2011). Nevertheless, the knowledge about the development of the microbial community, including *Bacteria*, *Archaea* and *Fungi*, in a rice-maize crop rotation system is limited and has to be improved by investigating rice-maize crop rotation soils at different locations and by analysing the influence of crop rotation on the bulk soil and rhizosphere microbiota. The development of the microbial community is especially of interest for rice-maize crop rotation systems in order to reduce methane emissions and face the increasing water demands.

1.4. Rhizosphere as habitat for microbes

A crop rotation between rice and maize will not only lead to different soil properties due to the alteration from a flooded to a non-flooded system, but also due to changed soil characteristics, because of the different characteristic physiologies of the plants and their individual rhizodeposition patterns (Breidenbach et al., 2015). Each plant species possesses individual root exudates, and releases a different blend and a substantial amount of carbon into the soil via rhizodeposition (Bais et al., 2006). Microorganisms inhabiting the rhizosphere are largely controlled by these carbon compounds (Haichar et al., 2008). Thus, the rhizosphere microbiota of different plant species is distinct (Berg and Smalla, 2009). The seasonal changes due to crop rotation can therefore lead to a stimulation of specific taxa and are likely to contribute to alternations in the soil microbial community and, therefore, may change the seasonal pattern of carbon and nutrient cycling in the soil (Lu et al., 2004).

The rhizosphere is defined as soil zone adjacent to and influenced by the roots of plants (Bais et al., 2006). In this region plant roots must compete with the invading root systems of neighbouring plants for space, water and mineral nutrients as well as with other soil organisms, including bacteria, fungi and insects feeding on the abundant source of organic material (Haichar et al., 2014). These interactions can be positive, including symbiotic associations with epiphytes and mycorrhizal fungi or root colonisation by plant growth-promoting bacteria (PGPR) (figure 1.2). Negative interactions can be competition or parasitism among plants, pathogenesis by bacteria or fungi and invertebrate herbivory (Bais et al., 2006). All these interactions are based on root-derived chemicals.

Plants are able to secrete a wide range of rhizodeposits. Rhizodeposits can be classified into four categories: i) secretions, considered as compounds actively released as a result of metabolic processes including antimicrobial, insecticide and nematicide compounds ii) lysates include compounds released by autolysis of older rhizodermal cells iii) border cells that detach from roots as they grow and iv) root exudates, which are compounds passively leached from internal root tissue into the soil matrix (figure 1.2) (Bais et al., 2006; Baudoin et al., 2001; Haichar et al., 2014).

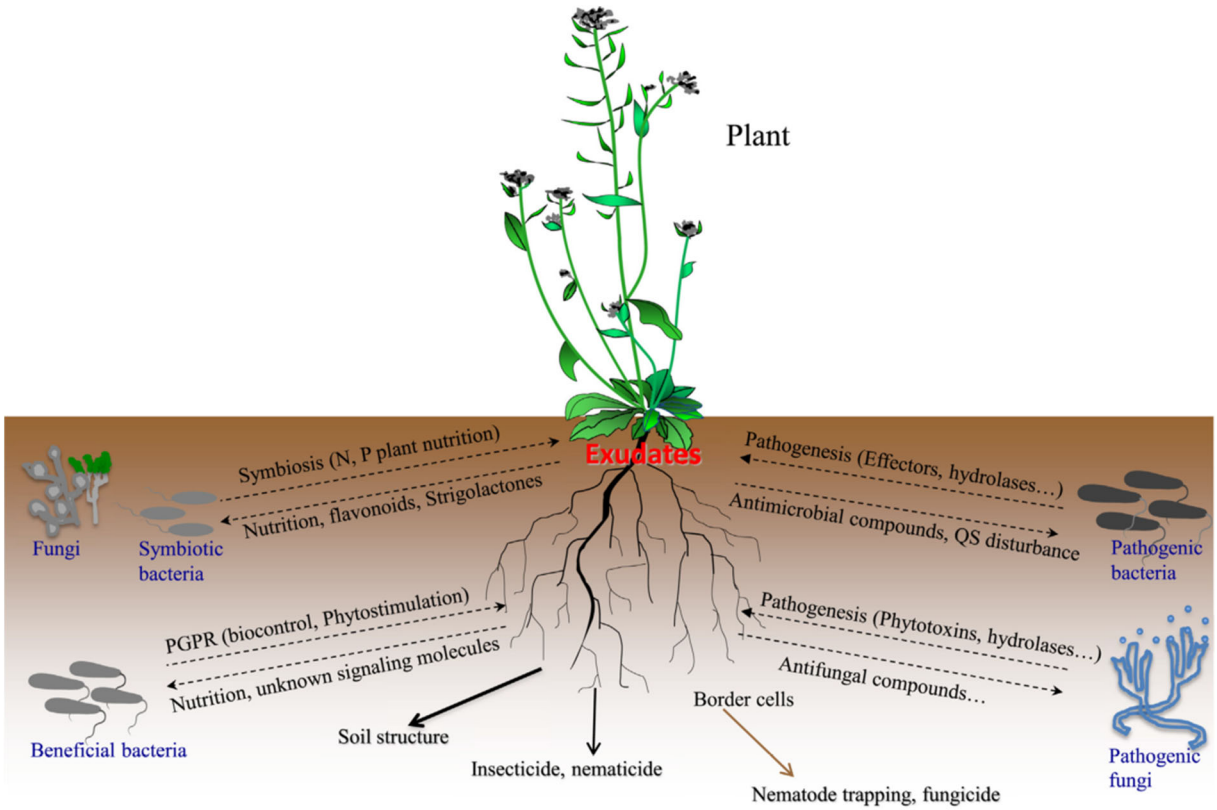


Figure 1.2: Interaction between plant and the rhizosphere microorganisms mediated by rhizodeposits. Positive interactions are represented on the left side and negative interactions are shown on the right side. PGPR: Plant growth promoting rhizobacteria, N: Nitrogen, P: Phosphate, QS: Quorum sensing (Haichar et al., 2014).

The passive leaching of root exudates is driven by i) diffusion due to a concentration gradient between root and soil ii) the permeability of the plasma membrane of the plant and iii) the spatial location of the solutes in the root tissue (e.g. epidermis versus stem) (Jones et al., 2009).

Root exudates (figure 1.3) contain compounds of low molecular weight (amino acids, organic acids, sugar phenolic and other secondary metabolites) and high molecular weight (mucilage (polysaccharides) and proteins) (Bais et al., 2006). About 5 – 40% of the total photosynthetically fixed carbon is transferred into the rhizosphere through root exudates (Bais et al., 2006; Derrien et al., 2005; Haichar et al., 2008; Nguyen, 2003) and between 40 and 90% of the excreted carbon is metabolized by root-associated microorganisms (Lynch and Whipps, 1990). According to that, root exudates are efficiently used as carbon source by rhizosphere inhabiting microorganisms and, thus, induce a rhizosphere priming effect (PE). This PE supports microbial degradation of organic matter and results in a specific rhizosphere microbiota compared to bulk soil samples (Baudoin et al., 2002; Berg and Smalla, 2009; Haichar et al., 2014).

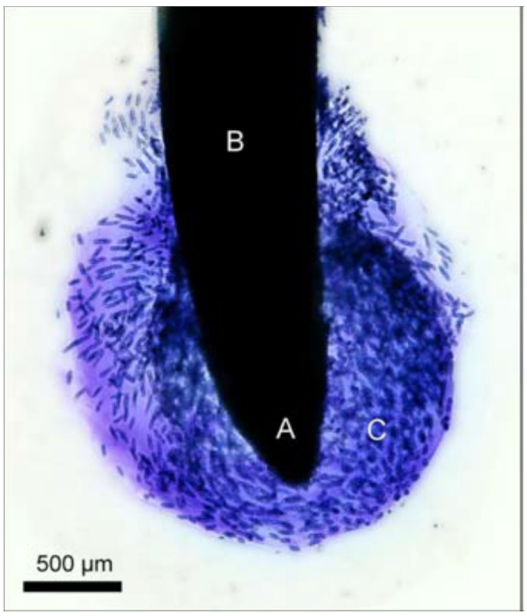


Figure 1.3: Light microscopic image showing the large amount of root exudates (blue halo surrounding the roots) and border cell production on a *Zea mays* root tip. A) Indicates the root centre, B) the main root elongation zone and C) the mucilage halo in which the border cells are embedded (Jones et al., 2009).

The amount and chemical composition of exudates differs depending on various factors including plant species, plant age and environmental conditions (Badri and Vivanco, 2009; Bais et al., 2006; Baudoin et al., 2001). These varying types and quantities of exudates influence the density and diversity of rhizosphere microorganisms and indicate a close evolutionary link between rhizosphere microbiota and plant species (Badri and Vivanco, 2009). The rhizosphere microbiota in turn plays an important role in the mobilisation or mineralisation of soil organic matter and is therefore responsible for modulating soil fertility (Kusliene et al., 2014). Therefore, the interaction between plant and microorganisms plays a central role in carbon and nutrient cycling in the rhizosphere (Lu et al., 2004). Nevertheless, Haichar et al. (2008) observed that not only plant specific species were involved in the consumption of exudates, but also some generalists, which can be detected as active exudate feeders in the rhizosphere of four different plants (maize, wheat, rape and barrel clover).

A close interaction between rice plants and microbes was also reported in paddy soils. In contrast to the bulk soil, the rice rhizosphere is characterized by the availability of oxygen. Oxygen diffuses via the rice aerenchym into the root zone, thus providing a habitat for both, anaerobic and aerobic microorganisms, which can inhabit the rice rhizosphere. The O_2 concentration decreases with increasing distance from the roots, because of the lower root density and consequently lower availability of root derived O_2, while the methane availability increases with distance from the rice roots (Shrestha et al., 2008). Due to the simultaneous presence of O_2 and methane, the rice rhizosphere provides an optimal habitat for methane oxidizing bacteria and is therefore the most important methane sink in rice fields. With

increasing distance from the roots other oxidized chemical compounds like sulphate and ferric iron are used as electron acceptors. This leads to the occurrence of genera like *Geobacter* or *Pelobacter* as well as of members of the families *Desulfobacteriaceae, Desulfovibrionaceae* (Scheid et al., 2004; Scheid and Stubner, 2001). Additionally, methanogens can be observed in the rhizosphere in regions where O_2 is more limited, where they consume plant-derived carbon compounds like acetate, which plays a key role in CH_4 production (Breidenbach et al., 2016; Lu and Conrad, 2005).

While the different oxygen availability in the bulk soil and rhizosphere of rice causes additionally different conditions, the availability of oxygen in the rhizosphere of maize is quite the same as in the bulk soil. Therefore the main impact factor shaping the rhizosphere microbiota of maize is the additional nutrient supply by the root exudates. Li et al. (2014) observed that the rhizosphere of maize is preferentially colonized by *Proteobacteria*, *Bacteriodetes* and *Actinobacteria*, with one or two dominating subsets of bacterial groups in each phylum. However, several studies observed that maize has different specific bacterial communities, depending on parameters like soil properties, genotype, crop management (such as fertiliser application) and growth stage (Aira et al., 2010; Baudoin et al., 2001; Castellanos et al., 2009; Li et al., 2014; Peiffer et al., 2013). These different parameters influence the quality and composition of root exudates, leading to differences in the composition of the rhizosphere microbiota (Aira et al., 2010; Baudoin et al., 2003; Butler et al., 2003). At earlier growth stages genera like *Massilia*, *Flavobacterium* and *Arenimonas* were observed at high abundance, while *Bulkholderia*, *Ralstonia*, *Dynella*, *Sphingobium*, *Bradyrhizobium* and *Varivorax* were dominant in the rhizosphere of maize at later growth stages (Li et al., 2014). The maize rhizosphere is also a habitat for diverse fungi, especially endophytes including arbuscular mycorrhizal fungi (Na Bhadalung et al., 2005; Wang et al., 2006), whereas the rhizosphere of rice as a flooded and partly anoxic habitat is less important for fungi (Murase et al., 2006).

Due to the fact that rhizodeposits have a high impact on the rhizosphere microbiota, it is presumable that changes in cultivation systems like the introduction of crop rotation will have an impact on the rhizosphere community composition and diversity. In a soil under crop rotation a specific rice rhizosphere microbiota will develop during rice cultivation and a specific maize rhizosphere microbiota will develop during maize cultivation. Those different communities will partly remain in the soil, which could also have an impact on the next generation of the rhizosphere microbiota. However, it is still unclear whether the introduction of crop rotation will induce specific microbial responses in the bulk soil and rhizosphere of maize.

1.5. Agricultural aims of rice straw application

As crop rotation results in an increasing nitrogen, carbon and water loss due to the formation of desiccation cracks, rice straw application is a common method to compensate these losses (Asari et al., 2007; Cabangon and Tuong, 2000). Especially the use of rice straw as mulch after drainage of the rice fields helps to conserve the moisture in the soil and therefore compensates the high water loss and the development of desiccation cracks (Cabangon and Tuong, 2000).
Due to rice straw application, a total organic matter supply of 60 – 90 % is returned to the field (Kimura et al., 2004). The addition of rice straw as an additional carbon source can induce a PE. The PE is the stimulation of the decomposition of soil organic matter (SOM), following the supply of energy rich fresh organic matter (FOM) (Kuzyakov, 2010; Pascault et al., 2013).

Such FOM inputs create hotspots of microbial activity, in which the turnover rates of carbon are much higher than outside of these zones and where an acceleration of the degradation of SOM can be observed (Fontaine et al., 2003; Kuzyakov, 2010). The lifetime of such hotspots is limited until the FOM is degraded (Pausch and Kuzyakov, 2011).
The quality of FOM influences the composition of the FOM- and SOM-degrading communities strongly, with important consequences on PE intensity and thereby mineralization of soil organic C (Pascault et al., 2013). FOM, which is readily degradable, offers optimal growth conditions and due to that an increase in microbial biomass (Pascault et al., 2013). The resulting community can synthesize more exoenzymes to make FOM-substrate available, which may also help to decompose SOM and thus stimulate the SOM degrading community. Readily degradable FOM is decomposed by copiotrophic organisms. Copiotrophics preferentially consume labile soil organic C, have high nutrient requirements and can exhibit high growth rates when resources are abundant (Fierer et al., 2007). The relative abundance of those organisms increases with the availability of easy degradable FOM and decreases with time and increasing availability of recalcitrant FOM compounds during the decomposition process (Bernard et al., 2007; Pascault et al., 2013). A rapid and durable stimulation of copiotrophic organisms was observed previously after addition of straw, e.g. taxa of the classes *Beta*- and *Gammaproteobacteria* (Bernard et al., 2007; Pascault et al., 2013). In contrast, oligotrophic microorganisms like *Acidobacteria*, *Deltaproteobacteria* and fungi such as *Basidiomycota* (Bastian et al., 2009; Fierer et al., 2007) exhibit slower growth rates and are likely to outcompete copiotrophs under conditions of low nutrient availability due to their higher substrate affinities (Fierer et al., 2007). Bastian et al. (2009) confirmed this by their observation of an increased abundance of oligotrophic microorganisms during the decomposition process of straw, along with a decline in substrate quality and quantity over time. In summary, the PE depends on the

interactions between copiotrophic and oligotrophic organisms. The succession of microbial FOM degraders is an important driver of the PE and, therefore, of the carbon dynamics in soils (Pascault et al., 2013).

The degradation process of straw also depends on the environmental and agricultural conditions. So, this process differs between flooded rice soils and non-flooded maize soils. The main differences occur due to the availability of oxygen. The degradation process of straw differs between oxic and anoxic soils. In rice fields the decomposition of straw leads to an increased CH_4 production and a changed bacterial and archaeal community composition (Conrad et al., 2012a; Glissmann and Conrad, 2002; Shrestha et al., 2011). A complex microbial assemblage is involved in the anaerobic straw decomposition process consisting of hydrolytic, fermenting, homoacetogenic, syntrophic and methanogenic microorganisms (Rui et al., 2009). The first step of the degradation process under anaerobic conditions is the hydrolysis of polysaccharides by extra-cellular enzymes. The released monomers are further degraded by fermentation to fatty acids, alcohols, CO_2 and hydrogen (H_2) and a syntrophic conservation of fatty acids and alcohols to acetate, resulting in an accumulation of H_2 and acetate. Hydrogen is than consumed by respiratory processes (e.g. sulfates (SO_4^{2-}) reduction), so that hydrogenotrophic methanogenesis sometimes becomes substrate limited, but acetate is still accumulated and can be utilized by acetoclastic methanogens (Conrad et al., 2012b, 2012a; Glissmann and Conrad, 2002). Further, Conrad et al. (2012b) detected that straw degradation causes a negative priming effect on the methanogenic degradation of soil organic matter. Meaning that less SOM was utilized for CH_4 production when straw was available as substrate (Conrad et al., 2012b). Besides methanogens, several bacterial taxa like *Firmicutes* (mainly *Clostridia* and *Negativicutes*), *Actinobacteria*, *Spirochaetes*, *Bacteriodetes*, *Chlorobi* and *Proteobacteria* have been detected as straw degraders in rice soils (Asari et al., 2007; Lee et al., 2011; Rui et al., 2009). Rui et al. (2009) postulate that taxa that appear at the early stage of straw application like *Clostridia* are responsible for the degradation of easily degradable fractions of straw, whereas members of *Bacteriodetes* and *Chlorobi* were abundant in later successional stages and may be more important for the decomposition of complex straw compounds. Several studies indicate that *Clostridia* are mainly involved in the straw degradation process in bulk soil as well as in the rice rhizosphere (Lee et al., 2017; Shrestha et al., 2011). The rice rhizosphere as an oxic-anoxic interface provides different conditions compared to the anoxic bulk soil, thus microorganisms responsible for straw degradation in the rhizosphere differ from those in bulk soil (Shrestha et al., 2011). Besides *Clostridia,* mainly methanogens like *Metharnosarcina* and *Methanobacteriaceae* are dominant straw degraders in the rice rhizosphere, whereas *Proteobacteria*, *Actinobactera* and *Bacteriodetes,* which are

responsible for straw degradation in bulk soil, could not be detected in the rhizosphere (Shrestha et al., 2011). Thus, the additional input of carbon by straw enhances the abundance of anaerobic microorganisms, especially methanogens and, therefore, increases methane production and emissions in paddy fields, which is a further problem in the context of global warming.

In comparison to anoxic soils, the composition of straw degrading microbes under oxic conditions is largely different. In oxic soils rice straw derived carbon compounds are primarily oxidised to CO_2, while under anoxic conditions mainly CH_4 is produced. Even in drained paddy soils, a growth of methanogens can be observed after straw addition (Lee et al., 2012). Nevertheless, it can be assumed that different microbial communities are responsible for the degradation of straw in fields managed under different water regimes (Lee et al., 2017).

In contrast to paddy soils, strictly anaerobic straw degraders like members of *Clostridia*, *Negativicutes* or methanogens cannot be found in oxic soils. Gram-positive bacteria, which belong to *Actinobacteria* as well as *Bacilli,* are dominant members of the straw degrading community in rice soils under oxic conditions (Lee et al., 2011, 2017). However, some members of Gram-negative bacteria like *Proteobacteria* and *Bacteriodetes* are commonly found under both conditions (Bernard et al., 2007; Lee et al., 2017). In oxic soils, the fungal community is also involved in the degradation of plant residues, but depending on the quality and composition of plant residues, different fungal taxa are stimulated (España et al., 2011; Nakamura et al., 2003). Previous studies observed that slow growing fungal decomposers (i.e. *Penicillium*, *Aspergillus*) were stimulated after addition of maize residues, while relatively fast-growing fungi (i.e. *Fusarium*, *Mortierella*) were dominant after addition of soybean residues (España et al., 2011; Murase et al., 2012). In contrast to oxic soils, a stimulation of fungi by straw addition in flooded soils was not observed, presumably due to the mainly anoxic conditions (Kimura et al., 2001; Nakamura et al., 2003).

The different conditions in agricultural soils influence the process of straw decomposition and therefore the straw degrading community. The straw degrading community in paddy soils as well as in upland soils has already been studied as mentioned before, but until now it has not been investigated what kind of microorganisms are responsible for straw decomposition in soils undergoing a rice-maize crop rotation and how this community develops due to the continuous changes between flooded and non-flooded conditions. Furthermore, it is unknown whether the application of straw in crop rotation soils impacts the microbial community in the rhizosphere of maize to the same extent as in bulk soil and whether the same microorganisms are involved in the straw degradation process in the different compartments. Additionally, it has not been

investigated so far whether straw addition impacts the uptake of root exudates by rhizosphere microorganisms in a rice-maize crop rotation system.

1.6. Aims of the study

Methanotrophic bacteria represent a methane sink in the different habitats of rice fields. Previously, methanotrophs were mostly detected by cultivation-independent methods in the rice soil, in the rice rhizosphere and in the rice phyllosphere (Conrad, 2007; Iguchi et al., 2012; Knief et al., 2012; Lüke et al., 2010), but the cultivation of methanotrophs remains challenging. Despite the occurrence of methanotrophs in rice fields, most of the methane produced in rice field is emitted into the atmosphere. Therefore, alternative field management strategies have to be developed to reduce the methane emissions. One prevention strategy is the introduction of upland maize into a rice monoculture. However, this agricultural management practice results in a lowered release of CH_4, but causes a great loss of C, N and water. To counteract these problems, rice straw is applied to fields under crop rotation.

It is known that agricultural management practises like crop rotation or straw addition influence the microbial community composition, but how the introduction of both practises in a rice paddy system influences the microbial community is unclear. The introduction of a crop rotation regime between flooded rice and non-flooded maize leads to dramatic changes in field conditions, because of the completely different redox conditions and the different rhizosphere microbiota of rice and maize that will partly remain in the soil. These aspects will induce a specific microbial response. But it is unknown to what extent the change of the flooding regime and the plant variation between rice and maize impacts the microbial communities in bulk soil as well as in the rhizosphere of maize.

The application of straw in differently managed fields causes changes in microbial community composition and diversity in rice and maize field soils, respectively. It stimulates the activity and growth of specific taxa that benefit from the supply of FOM. Several previous studies observed an increased CH_4 production in flooded rice fields and an increased CO_2 production in maize fields after straw addition. Even in drained paddy fields, which were managed under rice monoculture, a growth of methanogens was detected (Asari et al., 2007; Conrad et al., 2012b; Lee et al., 2012, 2017; Rui et al., 2009; Shrestha et al., 2011). Based on these observations the following hypotheses arise and the research areas are summarized in figure 1.4.

1. The cultivation of aerobic methanotrophic bacteria from the phyllosphere of rice is possible. Therefore, this compartment represents a potential methane sink in rice systems (**manuscript 1**).
2. The introduction of a rice-maize crop rotation regime affects the bacterial, archaeal and fungal community in the bulk soil and rhizosphere of maize. The microbial community in soil under crop rotation will be more similar to a community typical for rice monoculture soils than to a community typical for maize monoculture soil (**manuscript 2**).
3. The straw application in soils with different cultivation history (rice monoculture, maize monoculture or rice-maize crop rotation soil) will cause changes in microbial communities (**manuscript 2**). In rice-maize crop rotation soils with straw application mostly aerobic microorganisms will degrade the straw during maize cultivation because of the predominantly non-flooded conditions. Further, a specific response due to straw addition on the microbial community can be expected in the bulk soil and rhizosphere of maize (**manuscript 3**).
4. The rhizosphere microbiota is influenced by root exudates of maize. Nevertheless, an effect of straw addition on the rhizosphere microbiota is expected and leads to an altered uptake of root exudates by microbes, because of the additional benefit from straw (**manuscript 2, 3 & 4**).

This thesis resulted as part of a scientific consortium answering the question if rice-maize crop rotation in combination with straw addition can significantly lower greenhouse gas emissions and reduce water loss during maize cultivation. Therefore, the analyses of microbial community shifts due to these management strategies were mandatory.

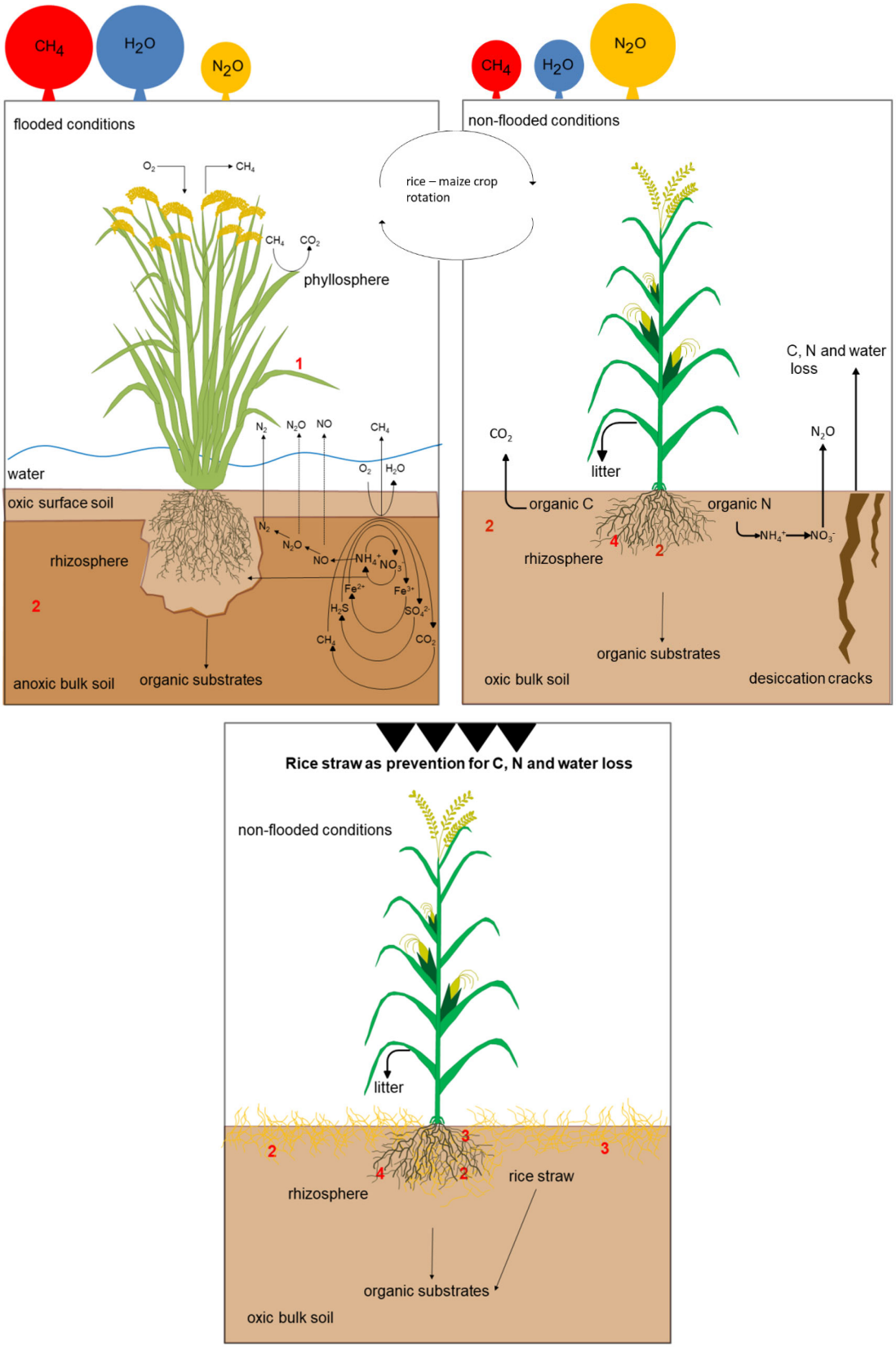


Figure 1.4: Graphical summary of the research objects presented in this doctoral thesis. Numbers represent the single manuscripts and correspond to the chapters in the results section. The experiments corresponding to number 1 were performed in the rice phyllosphere. Investigations for the second manuscript were conducted in bulk soil and rhizosphere of rice monoculture soil, maize monoculture soil (not included in this figure) and rice-maize crop rotation soil treated with and without straw. Experiments corresponding to number 3 were performed in the bulk soil and rhizosphere of rice-maize crop rotation soil treated with straw, and the experiments corresponding to manuscript 4 were conducted in the rhizosphere of maize in a rice-maize crop rotation soil treated with and without straw.

2. Results

2.1. Characterization of the first rice paddy cluster I isolate, *Methyloterricola oryzae* gen. nov., sp. nov. and amended description of *Methylomagnum ishizawai* (manuscript 1)

Katharina Frindte[1], Sarah A. Maarastawi[1], André Lipski[2], Joachim Hamacher[3] and Claudia Knief[1]

[1] University of Bonn, Institute of Crop Science and Resource Conservation, Molecular Biology of the Rhizosphere, Nussallee 13, 53115 Bonn

[2] University of Bonn, Institute of Nutritional and Food Sciences, Food Microbiology and Hygiene, Meckenheimer Allee 168, 53115 Bonn, Germany

[3] University of Bonn, Institute of Crop Science and Resource Conservation, Plant Diseases and Crop Protection, Nussallee 9, 53115 Bonn

Author contributions:

KF and SM performed the experiments. KF, SM, AL, JH and CK analysed the data. KF, SM and CK wrote the paper.

Published in International Journal of Systematic and Evolutionary Microbiology

Permission for reuse granted with the licence number: 4387180845039, the order detail ID: 71305698 and the confirmation number: 11730638.

Abstract

Three gammaproteobacterial methanotrophic strains (73a^T, 175 and 114) were isolated from stems of rice plants. All strains are Gram-negative, motile and grow on methane or methanol as sole carbon source. They oxidize methane using the particulate methane monooxygenase. Strains 114 and 175 possess additionally a soluble methane monooxygenase. All strains contain significant amounts of the cellular fatty acids C16:0, C16:1ω6c and C16:1ω7c, typical for type Ib methanotrophs. Characteristic for strains 114 and 175 are high amounts of C14:0 and C16:1 ω 6c, while strain 73a^T contains high quantities of C16:1ω5c. 16S rRNA gene sequence analyses showed that strains 114 and 175 are most closely related to *Methylomagnum ishizawai* (≥99.6 % sequence identity). Strain 73a^T is representing a new genus within the family *Methylococcaceae*, most closely related to *Methylococcus capsulatus* (94.3 % sequence identity). Phylogenetic analysis of the PmoA sequence indicates that strain 73a^T represents rice paddy cluster I (RPCI), which has almost exclusively been detected in rice ecosystems. The GC content of strain 73a^T is 61.0 mol%, while strains 114 and 175 have a GC-content of 63.3 mol%. Strain 73a^T (= LMG 29185, VKM B-2986) represents the type strain of a novel species and genus, for which the name *Methyloterricola oryzae* gen. nov., sp. nov. is proposed and a description provided. Strains 175 (= LMG 28717, VKM B-2989) and 114 are members of the species *Methylomagnum ishizawai*. This genus was so far only represented by one isolate, so that an emended description of the species is given.

Aerobic methanotrophic bacteria (MOBs) inhabit diverse environments. They are found in aquatic ecosystems like sediments or the water column of oceans and lakes, as well as in terrestrial habitats like upland soils, peatlands, rice fields or permafrost soils [1]. In most of these ecosystems, MOBs are abundant in redox transition zones, where methane enters from an anoxic and oxygen from the oxic zone. In rice fields, MOBs are present at this oxic-anoxic interface in the soil column, as well as in the rhizosphere of rice plants, where oxygen is diffusing from the atmosphere via the aerenchymatic tissue of the plant [2]. MOBs act as sink for methane in rice fields and reduce the release of this greenhouse gas to the atmosphere substantially [3, 4]. Recently published in situ methane consumption rates indicate methanotrophic activity on the emerged parts of rice plants [5], raising the question whether MOBs reside on and can be isolated from the above-ground parts of plants.

MOBs are often divided into type I (corresponding to *Gammaproteobacteria*) and type II (*Alphaproteobacteria*), whereby in particular type I MOBs are often further differentiated into type Ia to Id [1]. In rice field soils and the rice rhizosphere, members of well-known genera such as *Methylocystis*, *Methylosinus*, *Methylomonas*, *Methylosarcina*, *Methylobacter* or *Methylosoma* have frequently been detected. Besides, cultivation-independent studies have revealed the presence of several uncultured groups, including type Ia methanotrophs and in particular type Ib methanotrophs. Among the latter are methanotrophs of rice paddy cluster 1 (RPC1), which is quite consistently found in rice ecosystems [1, 6]. Remarkably, a rather low number of different genera of MOBs have been isolated from rice fields so far, encompassing mostly the common genera *Methylocystis*, *Methylosinus* and *Methylomonas* [7, 8]. Only recently, further novel taxa of type I MOBs were described, including *Methylogaea* (soil-water interface, Uruguay), *Methylomagnum* (rice rhizosphere, Japan) and the *Methylococcaceae* strains BRS-K6 (rice field soil, Bangladesh) and Sn6-10 (rice rhizosphere, India) [9–11]. However, in cultivation-independent studies, these were not very frequently detected [1], indicating that the isolation of representative taxa remains challenging.

In this study, we aimed specifically at the isolation of type I methanotrophs living in association with rice plants. For the enrichment of MOBs, the basal aerial parts of rice stems were collected from *Oryza sativa* subsp. *indica* cv. IR-72 or cv. Angelica grown in a paddy field at the International Rice Research Institute (IRRI) in Los Baños, Philippines. The stems were used for imprints on nitrate mineral salts (NMS, ATCC medium 1306) and ammonium mineral salts (AMS, ATCC medium 784) media agar plates [12], which were incubated at 28°C for 6 weeks under a 20 - 30 % [v/v] methane-containing atmosphere. To stimulate growth, a vitamin solution was added [7]. Individual colonies were repeatedly picked, restreaked and incubated

for 3 to 4 weeks until pure cultures were obtained. If isolation failed via this way, individual colonies were resuspended in liquid AMS or NMS medium and serially 10-fold diluted in microtiter plates. Upon growth in the plates for 5 to 7 days, an aliquot of cell suspension from the highest positive dilution was spread on an agar plate and incubated until colonies appeared. These were picked, restreaked, grown and evaluated for purity. Purity was assessed microscopically and by cultivation on Nutrient Broth and Reasoner's 2A agar. No growth was detected on these plates in case of pure cultures.

To differentiate the methanotrophic isolates, the pmoA gene sequence was analyzed, as it serves as a valuable molecular marker due to its broad occurrence in methanotrophic bacteria and its largely congruent phylogeny compared to the 16S rRNA gene [1]. To differentiate type I and II methanotrophic isolates, a restriction fragment length polymorphism analysis of pmoA PCR-products was performed. Cell biomass was transferred into a 0.2 ml PCR tube, filled with 100 µl of 50mM NaOH and 0.1 g of zirconium-silica beads of 0.1mm diameter. Cells were thermally lysed by incubating the suspension for 10 min at 99 °C, followed by mechanical lysis for 60 s in a bead beater (CapMixTM, 3M ESPE, Seefeld, Germany). Between 1 and 2 µl of cell extract were used as template in a PCR assay as described earlier [13]. Four µl of PCR product were digested overnight in 10 µl assays with BclI (Fermentas) and the band patterns visualized on 3 % agarose gels. The pmoA PCR products of *Methylocystaceae* were specifically cut at 290 bp. After classification into type I and II MOBs, the pmoA PCR products of type I isolates were sequenced. This revealed the isolation of *Methylococcus*, *Methylomonas* and some isolates without closely related cultivated strains. The latter were further differentiated at strain level based on BOX-PCR, as described earlier [14]. It revealed the presence of three different groups (Figure S1), of which strains 73a^T, 114 and 175 were selected as representatives and characterized in detail. In all subsequent experiments, strains 114 and 175 were cultured on AMS medium and strain 73a^T on NMS medium.

Cell morphology was assessed via bright field, fluorescence (Leica DMRB, Wetzlar, Germany) and phase-contrast microscopy (Zeiss Axio Imager M2, Jena Germany). Cell inclusions were analyzed microscopically via cell staining with nile blue for poly-β-hydroxybutyrate (PHB) and counterstaining with 4',6-diamidino-2-phenylindole (DAPI, 0.1 µg mL-1), which visualizes polyphosphate granules [15,16]. Electron microscopy was performed after embedding cells in a drop of 10 % agarose solution. Thin slices were fixed after [17] and stained with 1 % osmiumtetroxide solution. Microscopic preparations were observed under an EM 109 electron microscope (Zeiss).

Motility was tested by spotting cell suspension on a 10-fold diluted medium agar plate and observation of the spreading upon growth. Oxidase was tested with a 1 % N,N,N,N-tetramethyl p-phenylenediamine solution, while catalase activity was assessed by exposing cells to 3 % H_2O_2. Gram testing behavior was evaluated by placing a loop of fresh cell material into a drop of 3 % potassium hydroxide [18]. The growth of the strains on different carbon sources was tested by using a final concentration of 0.05 % of the following carbon compounds: acetate, succinate, methanol, ethanol, isopropanol, methylamine, formamide, formic acid, glycine, pyruvate, sucrose, fructose, glucose, ribose, urea, malic acid, fumaric acid, oxalic acid and yeast extract. The use of different nitrogen sources was tested with 1 and 10 mM of lysine, urea, sodium nitrate, ammonium chloride, formamide, methylamine, arginine, glycine, asparagine and glutamine. To test for nitrogen fixation potential, a nitrogen-free medium was prepared. Furthermore, salt tolerance [0.1, 0.2, 0.5 and 1 %], pH [2.3, 4.6, 5.0, 5.5, 6.1, 6.6, 7.1, 7.5, 7.8] and temperature range [4, 8, 15, 20, 27, 30, 37, 40°C, 45 and 50°C] were evaluated. To study the lysis behavior of strain 175, copper sulfate was added in concentrations of 0, 20, 40, 60 and 89 µM to liquid medium cultures and cell appearance was analyzed microscopically over time.

DNA from all strains was extracted using the MasterPure™DNA Purification Kit (Epicentre). The GC content was determined based on melt-curve analysis in a qPCR cycler (CFX Connect, Biorad) as described [19]. Phylogenetic classification was done based on 16S rRNA gene sequence analysis. The gene of strain 114 was PCR amplified and sequenced using the 9f/1492r primer set [20]. For strains 73a^T and 175, sequences were taken from draft genome sequence datasets [21]. 16S rRNA gene sequences were aligned using the SINA aligner [22], imported into the SSU Ref NR 99 database, the alignment manually controlled and a phylogenetic tree constructed in ARB. Likewise, a PmoA based phylogenetic tree was calculated in ARB using a customized PmoA database (Knief, 2015). Information about the presence of genes involved in one-carbon and nitrogen metabolism such as mmoX, mxaF, nifH as well as of the ribulose-1,5-bisphosphat-carboxylase (RuBisCO) and the ribulose monophosphate (RuMP)-pathway was taken from the draft genome sequence analysis [21].

Whole cell fatty acids were extracted from the three strains and the reference strains *Methylogaea oryzae* E10T (DSM 23452), *Methylococcus capsulatus* Bath (LMG 26900), *Methyloparacoccus murrellii* R 49797T (LMG 27482) and *Methylocaldum gracile* 14LT (NCIMB 11912) according to [23] and analyzed by GC-MS [24].

Two-week-old colonies of strain 73a^T grown on agar plates appeared white, round and slimy, while colonies of strains 114 and 175 were white and round. Older cultures of strain 73a^T were slightly pink, while colonies of strains 114 and 175 became beige-brownish. Cells of strain 73a^T

were small cocci with a size range between 1.6 and 1.9 µm x 1.2 and 1.4 µm (length x width). Strains 114 and 175 appeared as large short thick rods with a size range between 3.6 to 4.8 µm x 2.6 to 3.5 µm. Upon aging, cells of strains 114 and 175 became larger, very fragile and they divided incompletely, i. e. formed chains often consisting of four to six cells (Figure 1G), similarly as previously described for *Methylobacter chroococcum* strain G by Whittenbury et al. [12]; a strain that has been lost over time. The fragility of the aged cells could be reduced by increasing the copper concentration in the medium. After 6 weeks of growth, fragile and irregular shaped cells were found in media with copper concentrations of 0 - 40 µM, while cells in media with 60 µM and 89 µM copper appeared normally shaped. This may be linked to a higher membrane stability at higher copper concentrations, as proposed by for *Methylococcus capsulatus* [25].

All strains had intracytoplasmic membrane systems, which appeared as bundles of vesicular disks in transmission electron micrographs (Figure 1C, H and I); a characteristic feature of all type I MOBs [26]. Cell inclusions were clearly visible under the light microscope for strains 114 and 175. The number of inclusions increased in cells that had reached the stationary phase. They could be fluorescently stained in red with nile blue (Figure 1F) or in combination with DAPI in orange (Figure 1G), indicating the presence of PHB. In strain 73a^T, nile blue staining for PHB inclusion bodies was negative, while DAPI staining revealed the presence of polyphosphate (Figure 1B). In comparison, the draft genomes contain genes known to be involved in polyphosphate storage in both strains (73a^T and 175) and for PHB synthesis in strain 175 [21], suggesting that not all potential storage compounds were accumulated at the same time in these cells under the given conditions. Moreover, the genome data predict glycogen as storage compound in both strains.

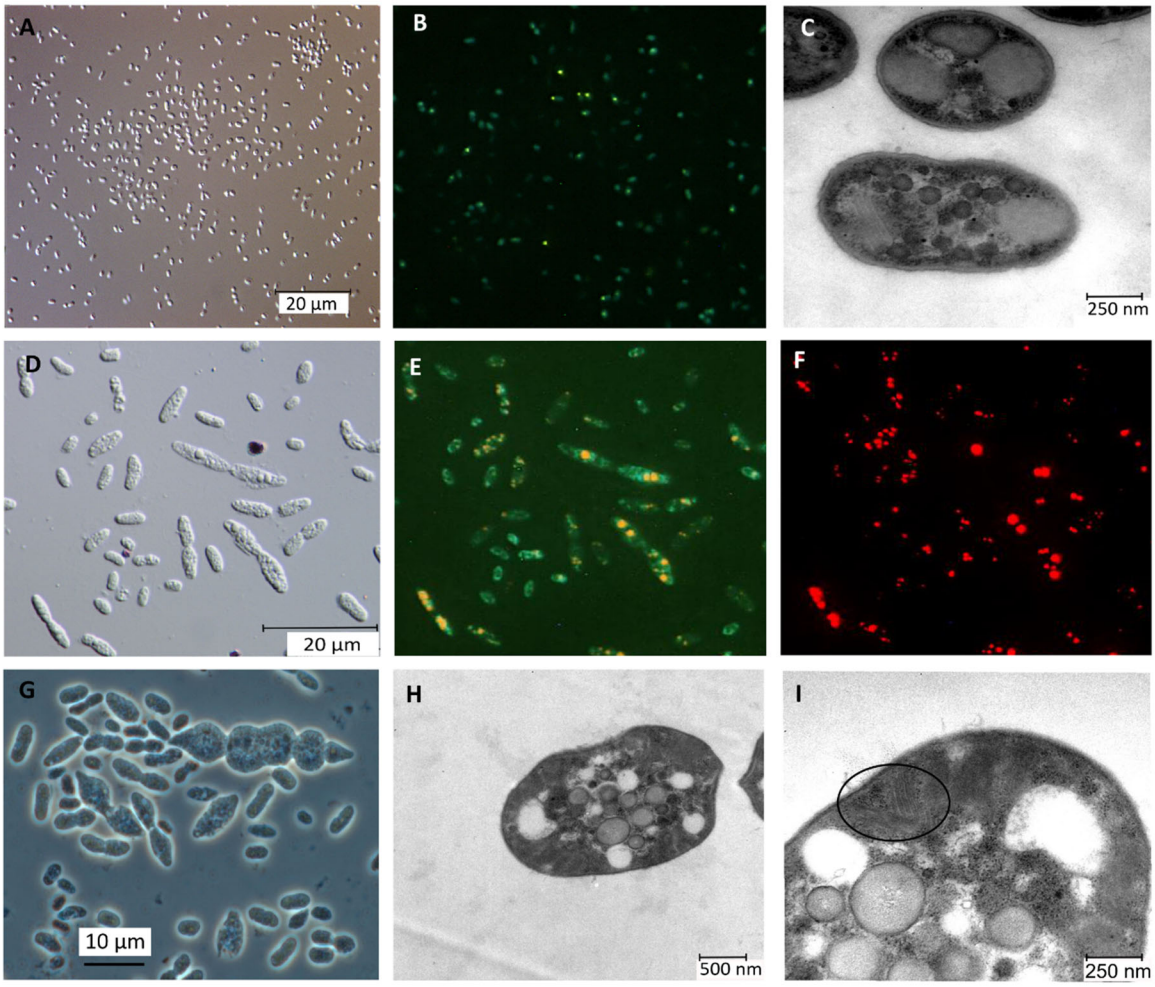


Figure 1: Photographs of strains 73a^T (A – C) and 175 (D-I) captured using bright field microscopy (A, D and G), epifluorescence microscopy (B, E, and F) and transmission electron microscopy (C, H and I). Cells of fresh cultures are shown in A and D (DIC objective), cells of a 6-week old culture of strain 175 are shown in G (phase contrast). DAPI staining revealed yellow stained polyphosphate granules in strain 73a^T (B). In strain 175, DAPI staining was negative, i.e. polyphosphate was not detected, while nile blue staining indicated the presence of PHB inclusion bodies (E and F; E shows the merged picture of DAPI and nile blue staining). The intracellular membrane system of strain 175 is highlighted by a circle (I).

The cells of all three strains were gram negative and motile. They exhibited no catalase activity and only 73a^T was cytochrome c oxidase positive. In comparison, the genome sequences revealed the presence of catalase in strain 175 and a cytochrome c oxidase in both strains [21].

Strains 73a^T and 175 grew only on methane and methanol as carbon sources, while strain 114 did not even grow on methanol under the given conditions. Besides the presence of a particulate methane monooxygenase, genome analysis revealed that strain 175 possesses a soluble methane monooxygenase [21]. Formaldehyde assimilation is predicted to occur via the RuMP pathway, likewise as in other type I MOBs. Moreover, genes encoding RuBisCO and enzymes involved in the Calvin cycle are present, suggesting that the strains may also use this pathway for carbon fixation [21]. The presence of RuBisCO has also been reported for other type Ib methanotrophs such as *Methylococcus* or *Methylocaldum* [27].

All isolates used urea, nitrate, ammonium, or formamide as nitrogen source and grew on nitrogen-free medium. In agreement with the latter observation, nif genes were present in the genomes of strains 73a^T and 175 [21]. Growth was also detected with 1 mM of glycine and glutamine as nitrogen sources, but not with 10 mM of these substrates. Strains 114 and 175 grew with up to 0.2 % sodium chloride, while strain 73a^T tolerated only 0.5 % salt in the growth medium. Strain 73a^T showed optimal growth within a pH range from 7.0 - 7.5 and at temperatures between 27°C and 37°C. Strains 175 and 114 grew best at pH values ranging from 6.0 - 6.6 and temperatures between 15°C – 37°C (Table 1). All three strains are thus mesophilic, supporting the recent statement that not all type Ib MOBs are specifically adapted to thermal habitats [28].

Table 1: Differential characteristics of strains 73a^T, 175, 114 and phylogenetically related type Ib methanotrophs Strains: 1, 73a^T; 2, 175 and 114; 3, *Methylomagnum ishizawai* RS11D-Pr^T; 4, *Methyloparacoccus murrellii* R-49797^T; 5, *Methylococcus capsulatus* Bath; 6, *Methylocaldum gracile* 14L^T; 7, *Methylogaea oryzae* E10^T; +, positive reaction; -, negative reaction; NA, not analyzed. Data for reference strains were taken from the following studies: 3, Khalifa et al. [10]; 4, Hoefman et al. [32]; 5, Bowman et al. [33]; 6, Bodrossy et al. [34]; 7, Geymonat et al. [9]. Temperature and pH values in brackets indicate optimal growth conditions.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Cocci / diplococcoid	Rods / diplobacilli	Rods	Cocci / diplococcoid	Cocci	Rods / pleomorph	Curved rods
Cell size (µm)	1.6-1.9 x 1.2-1.4	2.6-3.5 x 3.6-4.8	1.5-2.0 x 2.0-4.0	0.8-1.5	2.1-3.0 x 0.6-1.0	1.0-2.0 x 0.4-1.2	2.0-2.2 x 0.5-0.7
Chain formation	-	+	+	-	-	Variable	-
Mortility	+	+	+	-	Variable	+	-
PHB granula	-	+	+	+	NA	NA	+
Color	White	White to brownish	White	White	White to brownish or yellow	Brown	White
Growth temperature (°C)	15-45 (27-37)	4-37 (15-37)	20-37 (31-33)	20-37°C (25-33°C)	30-55 (37-50)	20-61 (42-55)	20-37 (30-35)
mmoX detection	-	+	+	-	+	-	-
Salt tolerance (%)	0.5	0.2	0.2	0.58	2.0	NA	0.5
nifH detection	+	+	-	-	+	NA	+
Growth on N-free medium	+	+	-	-	-	-	-
RuBisCo	+	+	+	NA	NA	+	NA
G + C content	61.0	63.6	64.1	65.6	62 – 65	56 – 60	63.1
PH	4.6-7.5 (7.0-7.5)	4.6-6.6 (6.0-6.6)	5.5-9.0 (6.8-7.4)	5.8-9.0 (6.3-6.8)	5.5-9.0	6.0-8.5 (7.0-7.2)	5.0-8.0 (6.5-6.8)
Reference	This study	This study	[10]	[32]	[33]	[34]	[9]

The cellular fatty acid composition of the strains revealed high abundances of C16 chain fatty acids, which are typical for most type I MOBs [1, 26, 29]. The strains contained high percentages of C16:0, especially in strain 73a^T it represented 30 % of all fatty acids (Table 2). All strains contained a significant proportion of C16:1ω7c, likewise as the other type Ib reference strains. High amounts (> 20 %) of C16:1ω5c and C16:1ω6c were characteristic for strain 73a^T (> 8 %). The strains 114 and 175 also contained C16:1ω6c (7 - 10 %) and a high percentage of C14:0 (> 30 %), which has so far only been reported for some *Methylomonas* strains [29]. In contrast to strain 73a^T, strains 114 and 175 showed more variability in their fatty acid composition in temporarily replicated experiments.

Table 2: Cellular fatty acid composition of strains 73a[T], 175, 114 and of reference strains from phylogenetically related genera Strains: 1, 73a[T]; 2, 114; 3, 175; 4, *Methylomagnum ishizawai* RS11D-PrT; 5, *Methylocaldum gracile* 14LT; 6, *Methylococcus capsulatus* Bath; 7, *Methylogaea oryzae* E10T; 8, *Methyloparacoccus murrellii* R-49797T; TR, traces (<1 %); numbers in brackets display the standard error of n=4 temporally independently cultured replicates. Data for strain 4 were taken from Khalifa et al. [10].

Cellular fatty acids	1	2	3	4	5	6	7	8
10:0		TR	TR[S]				0.6	0.7
13:0		TR						
14:0	TR	31.3 (3.0)	39.3 (3.6)	15.8	TR	3.4	0.7	TR
15:0	TR	10.4 (5.9)	2.7 (1.0)	1.6	15.3	28.6	1.3	44.0
15:1B		TR[S]			2.1			
16:0	30.9 (1.91)	23.6 (2.0)	30.5 (5.9)	19.6	44.7	34.3	33.2	15.2
16:0-OH				1.8				
16:1ω11c				5.5				
16:1ω10c	2.4 (2.4)[S]							
ECL 15.74475		TR	TR				5.2	
16:1ω7c	26.9 (1.4)	23.2 (4.8)	19.3 (2.4)	47.3	36.0	12.7	52.9	28.8
16:1ω6c	8.7 (0.3)	10.5 (4.3)	7.5 (2.1)	8.0		7.8		
16:1ω5c	28.3 (1.2)					6.4		
17:0					TR	1.1		
17:0 cyclo						TR		
17:1ω8c						TR		1.6
18:1ω7c	1.7 (1.2)							
Growth temperature (°C)	30	30	30	30	40	40	30	30

Phylogenetic analysis of the 16S rRNA gene confirmed that all three strains are *Gammaproteobacteria*, representing type Ib MOBs. While the sequences of strains 114 and 175 were closely related to sequence of *Methylomagnum ishizawai* RS11D-PrT (maximum 0.4 % sequence difference), strain 73a^T clustered distinctly from other type Ib type strains (Figure 2).

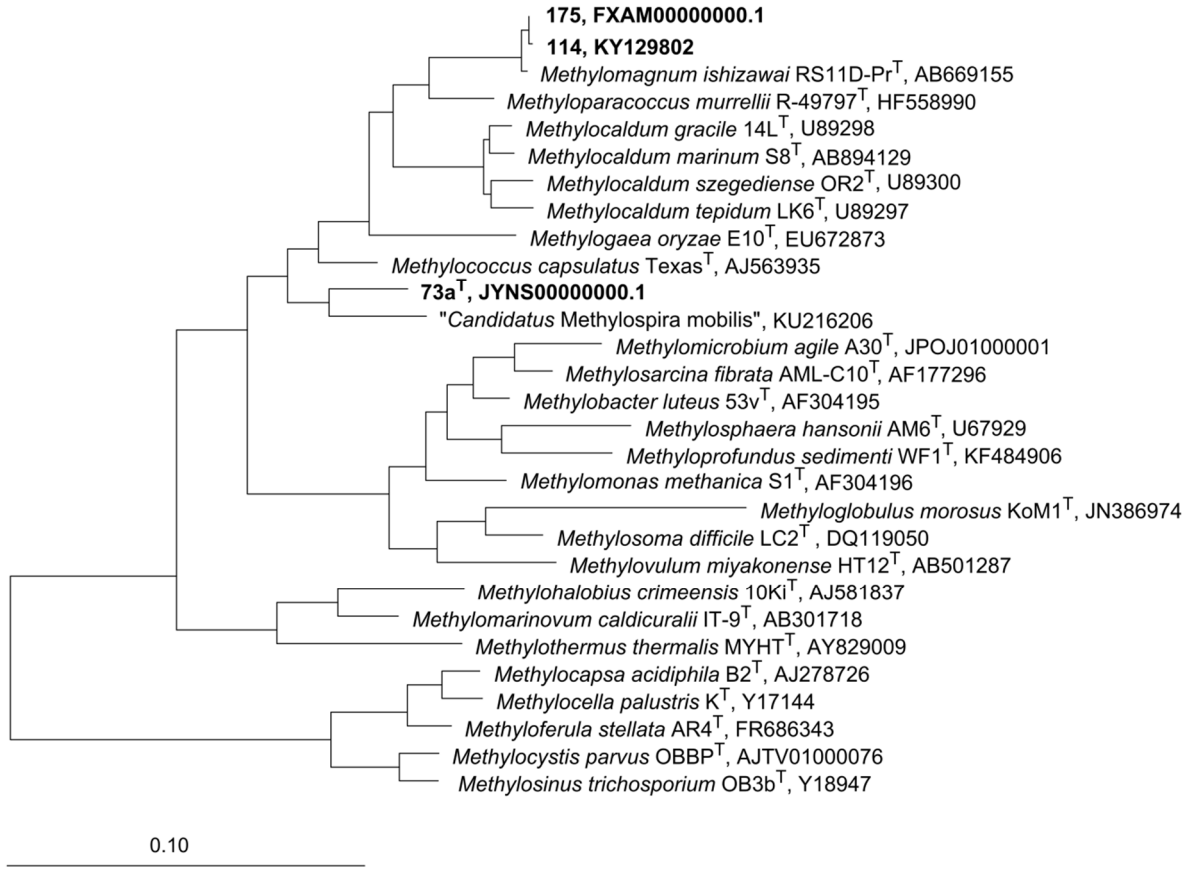


Figure 2: Phylogenetic tree based on 16S rRNA gene sequences, calculated using the maximum-likelihood algorithm PHYML and describing the phylogenetic position of strains 73a^T, 114 and 175. 16S rRNA gene sequences of *Methanosaeta* species were used to root the tree, but were excluded from the picture. The bar indicates 0.1 substitutions per nucleotide position.

The next cultured relatives were *Methylococcus capsulatus* with a sequence identity of 94.3 %, *Methylocaldum szegediense* (91.5 %), *Methyloparacoccus murrellii* (91.2 %) and *Methylomagnum ishizawai* (91.1 %). PmoA analysis largely confirmed the results of the 16S rRNA gene sequence analysis and indicated that strain 73a^T is an isolate of the RPC I cluster (Figure 3). This cluster is found with broad distribution in Eurasia, and almost exclusively in paddy soils [1,6,30,31]. The PmoA sequence of strain 73a^T was most closely related to *Methyloparacoccus murrellii* (85.8 %) and *Methylococcus capsulatus* (85.4 %). The PmoA sequences of strains 114 and 175 showed no or only minor differences (2.8 %) to the amino acid sequence of *Methylomagnum ishizawai*. Genome analysis revealed that strains 73a^T and 175 possess the pmoA-related gene pmxA [21]. Remarkably, the PmxA sequences were most

closely related to that of *Methylocystis rosea*, rather then to sequences of other type I MOBs (Figure 3).

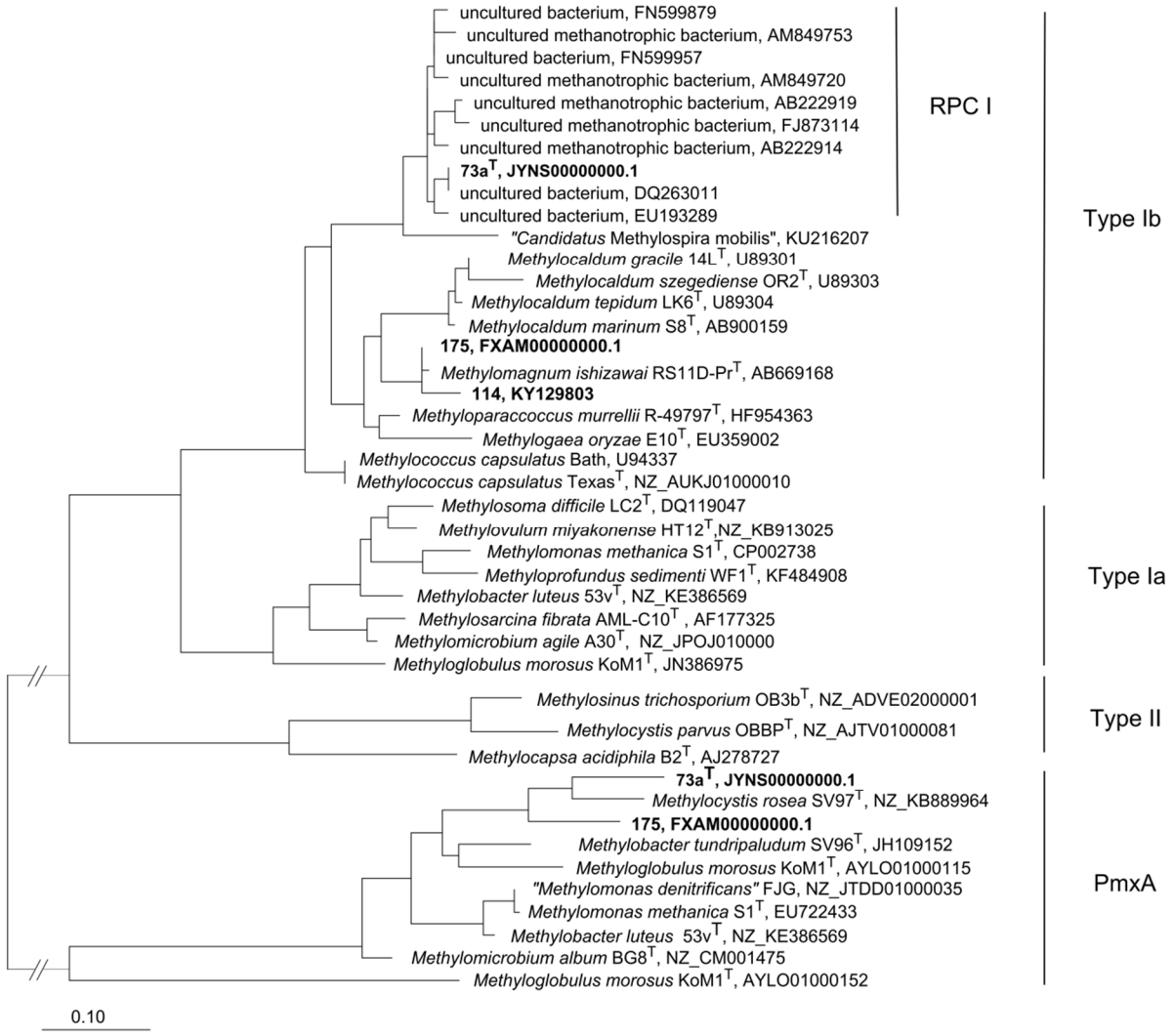


Figure 3: Phylogenetic tree of PmoA sequences, constructed using the maximum-likelihood algorithm PHYML with the JTT substitution model. PmxA sequences were used to root the tree. The bar indicates 0.1 substitutions per amino acid position.

Description of *Methyloterricola* gen. nov.

Methyloterricola (Me.thy.lo.ter.ri'co.la. N.L. n. methylum the methyl group; N.L. pref. methylo- pertaining to the methyl radical; L. fem. n. terra earth; LO. Suff. -cola (from L.n. incola) inhabitant; N.L. fem. n. *Methyloterricola* a terrestrial methyl-using bacterium). Cells are gram negative, aerobic, motile cocci. They show the typical intracellular membrane structures described for type I MOBs. The genus is obligate methanotrophic and cells can only use methane or methanol as carbon source. They are able to fix nitrogen and perform methane oxidation using the particulate methane monooxygenase. The major fatty acids are C16:1ω5c, C16:1ω6c and C16:1ω7c. The genus is most closely related to *Methylococcus* and

Methylocaldum in the class of *Gammaproteobacteria*. The type species is *Methyloterricola oryzae*.

Description of *Methyloterricola oryzae* sp. nov.

Methyloterricola oryzae (o.ry' zae. L. fem. n. *Oryza* genus name of rice; L. gen. n. *oryzae* of rice), referring to the fact, that the type strain was isolated from rice stems). Additionally to the genus description, the type strain displays the following properties. Cells have a size of 1.6 µm - 1.9 µm x 1.2 µm - 1.4 µm. The strain grows on NMS medium supplemented with urea, nitrate, ammonium, formamide, glycine or glutamine as nitrogen source. The growth temperature is between 15°C and 45°C (optimum is between 27 °C and 37 °C) and optimum pH ranges from 7.0 to 7.5. The strain is salt tolerant up to 0.5 %. The GC content is 61.0 %. Strain 73a^T (= LMG 29185, VKM B-2986) is the type strain and was isolated from rice stems of a rice field at the Philippines.

Amended description of the species *Methylomagnum ishizawai*

The size of the cells of this genus extend to a range between 1.5 µm and 3.5 µm x 2.0 µm and 4.8 µm. Cells in the stationary phase increase in size, accumulate different types of storage granula and can form cell chains. Cells can become very fragile when grown in standard media, a phenomenon that can be compensated by increasing the copper concentration. Cells have the ability to produce PHB and potentially polyphosphate and glycogen as storage compounds. They grow optimal between 15°C and 37°C and have an optimum pH range between 6.0 and 7.4. Some but not all strains possess nif genes for nitrogen fixation. Besides using the RuMP pathway for formaldehyde fixation, the strains have the potential to assimilate CO2 via RuBisCo and the Calvin cycle.

Acknowledgments

Merle Noschinski is acknowledged for excellent technical assistance and Lina Russ for setting up the pmoA based restriction analysis approach. We thank Julia Vorholt (ETH Zurich), who supported the initial phase of the project.

References

1. Knief C. Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. Front Microbiol 2015;6:1346

2. Conrad R. Microbial ecology of methanogens and methanotrophs. Adv Agron 2007; 96: 1–63.

3. Frenzel P. Plant-associated methane oxidation in rice fields and wetlands. Advances in microbial ecology 2000;16: 85–114.

4. Krüger M, Frenzel P, Conrad R. Microbial processes influencing methane emission from rice fields. Glob Chang Biol 2001 7: 49–63.

5. Yoshida N, Iguchi H, Yurimoto H, Murakami A, Sakai Y. Aquatic plant surface as a niche for methanotrophs. Front Microbiol 2014;5:30.

6. Lüke C, Krause S, Cavigiolo S, Greppi D, Lupotto E, et al. Biogeography of wetland rice methanotrophs. Environ Microbiol 2010;12: 862–872.

7. Heyer J, Galchenko VF, Dunfield PF Molecular phylogeny of type II methane-oxidizing bacteria isolated from various environments. Microbiology 2002;148: 2831–2846.

8. Ogiso T, Ueno C, Dianou D, Van Huy T, Katayama A, et al.. *Methylomonas koyamae* sp. nov., a type I methane-oxidizing bacterium from floodwater of a rice paddy field. Int J Syst Evol Microbiol 2012;62: 1832–1837.

9. Geymonat E, Ferrando L, Tarlera SE. *Methylogaea oryzae* gen. nov., sp. nov., a mesophilic methanotroph isolated from a rice paddy field. Int J Syst Evol Microbiol 2011; 61: 2568–2572.

10. Khalifa A, Lee CG, Ogiso T, Ueno C, Dianou D, et al. *Methylomagnum ishizawai* gen. nov., sp. nov., a mesophilic type I methanotroph isolated from rice rhizosphere. Int J Syst Evol Microbiol 2015; 65: 3527–3534. doi:10.1099/ijsem.0.000451.

11. Pandit PS, Rahalkar MC, Dhakephalkar PK, Ranade DR, Pore S, et al. Deciphering Community Structure of Methanotrophs Dwelling in Rice Rhizospheres of an Indian Rice Field Using Cultivation and Cultivation-Independent Approaches. Microb Ecol 2016;71: 634–644.

12. Whittenbury R, Phillips KC, Wilkinson JF. Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 1970; 61: 205–218.

13. Knief C, Lipski A, Dunfield PF. Diversity and Activity of Methanotrophic Bacteria in Different Upland Soils. Appl Environ Microbiol 2003;69: 6703–6714.

14. Knief C, Frances L, Vorholt JA. Competitiveness of diverse *Methylobacterium* strains in the phyllosphere of *Arabidopsis thaliana* and identification of representative models, including *M. extorquens* PA1. Microb Ecol 2010;60: 440–452.

15. Cain AJ. The use of Nile blue in the examination of lipoids. J Cell Sci 1947;3: 383–392.

16. Günther S, Trutnau M, Kleinsteuber S, Hause G, Bley T, et al.. Dynamics of polyphosphate-accumulating bacteria in wastewater treatment plant microbial communities detected via DAPI (4′, 6′-diamidino-2-phenylindole) and tetracycline labeling. Appl Environ Microbiol 2009;75: 2111–2121.

17. Karnovsky MJ. A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy. J cell Biol 1965;27: 137.

18. Buck JD. Nonstaining (KOH) method for determination of gram reactions of marine bacteria. Appl Environ Microbiol 1982;44: 992–993.

19. Gonzalez JM, Saiz-Jimenez C. A fluorimetric method for the estimation of G+ C mol% content in microorganisms by thermal denaturation temperature. Environ Microbiol 2002;4: 770–773.

20. Weisburg WG, Barns SM, Pelletier DA, Lane DJ 16S Ribosomal DNA Amplification for Phylogenetic Study 1991;173: 697–703.

21. Frindte K, Kalyuzhnaya MG, Bringel F, Dedysh S, Dunfield PF, et al.. Draft genome sequences of two gammaproteobacterial methanotrophs isolated from rice ecosystems. Genome Announc 2017;5e00526-17.

22. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 2012;28: 1823–1829.

23. Sasser M. Identification of bacteria through fatty acid analysis. Klement Z, Rudolph K, Sands DC, editors Budapest: Akademiai Kiado. 1990; 199-204 p.

24. Wiertz R, Schulz SC, Müller U, Kämpfer P, Lipski A. *Corynebacterium frankenforstense* sp. nov. and *Corynebacterium lactis* sp. nov., isolated from raw cow milk. Int J Syst Evol Microbiol 2013;63: 4495–4501.

25. Choi D-W, Kunz RC, Boyd ES, Semrau JD, Antholine WE, et al. The membrane-associated methane monooxygenase (pMMO) and pMMO-NADH: quinone oxidoreductase complex from Methylococcus capsulatus Bath. J Bacteriol 2003;185: 5755–5764.

26. Hanson R, Hanson T. Methanotrophic bacteria. Microbiol Rev 1996;60: 439–471.

27. Trotsenko YA, Murrell JC. Metabolic aspects of aerobic obligate methanotrophy. Adv Appl Microbiol 2008; 63: 183–229.

28. Danilova O V, Suzina NE, Van De Kamp J, Svenning MM, Bodrossy L, et al. A new cell morphotype among methane oxidizers: a spiral-shaped obligately microaerophilic methanotroph from northern low-oxygen environments. ISME J 2016;10:2734-2743.

29. Bowman JP, Skerratt JH, Nichols PD, Sly LI. Phospholipid fatty acid and lipopolysaccharide fatty acid signature lipids in methane-utilizing bacteria. FEMS Microbiol Lett 1991;85: 15 -21.

30. Jia Z, Kikuchi H, Watanabe T, Asakawa S, Kimura M. Molecular identification of methane oxidizing bacteria in a Japanese rice field soil. Biol Fertil Soils 2007; 44: 121–130.

31. Shrestha M, Abraham W-R, Shrestha PM, Noll M, Conrad R. Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. Environ Microbiol 2008; 10: 400–412.

32. Hoefman S, van der Ha D, Iguchi H, Yurimoto H, Sakai Y, et al. *Methyloparacoccus murrellii* gen. nov., sp. nov., a methanotroph isolated from pond water. Int J Syst Evol Microbiol 2014;64: 2100–2107.

33. Bowman JP, Sly LI, Nichols PD, Hayward AC. Revised Taxonomy of the Methanotrophs: Description of *Methylobacter* gen. nov., Emendation of *Methylococcus*, Validation of *Methylosinus* and *Methylocystis* Species, and a Proposal that the Family *Methylococcaceae* Includes Only the Group I Methanotrophs. Int J Syst Bacteriol 1994;44: 375–375.

34. Bodrossy L, Holmes EM, Holmes AJ, Kovács KL, Murrell JC. Analysis of 16S rRNA and methane monooxygenase gene sequences reveals a novel group of thermotolerant and thermophilic methanotrophs, Methylocaldum gen. nov. Arch Microbiol 1997; 168: 493–503.

Supplementary material

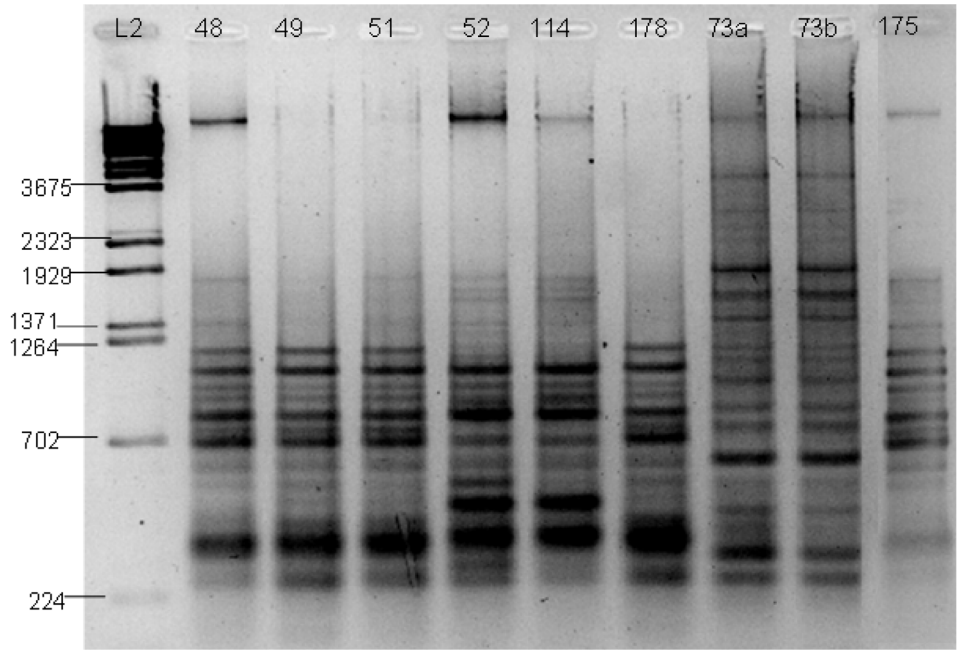


Figure S1: Box-PCR pattern of all type I methanotrophic isolates, which were distantly related to cultivated MOBs according to pmoA sequence analysis. Strains 48, 49, 51, 178 and 175 show very similar patterns, as well as strains 52 and 114 and strains 73a and 73b, suggesting that these are clonal isolates, respectively. Strains 114, 175 and 73a were selected as representatives from each of these groups for a detailed characterization characterization. A DNA size marker indicating molecule length in base pairs is shown on the first lane (L2).

2.2. Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of *Zea mays* (manuscript 2)

Sarah A. Maarastawi[1], Katharina Frindte[1], Marius Linnartz[1], Claudia Knief[1,*]

[1]Institute for Crop Science and Resource Conservation (INRES), Microbiology of the Rhizosphere, University of Bonn, Bonn, Germany

Author contributions:

SM, KF, and CK conceived and designed the research, and wrote the paper. SM performed the experiments. SM, ML, and CK analysed the data. All authors discussed the results and approved the paper.

Published in *Frontiers in microbiology* (2018) doi: 10.3389/fmicb.2018.01295

The Supplementary table 6 of this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01295

Abstract

Rice is one of the most important nourishments and its cultivation binds large agricultural areas in the world. Its cultivation leads to huge water consumption and high methane emissions. To diminish these problems, crop rotation between paddy rice and maize is introduced in Asia, but can lead to losses of carbon and water by the formation of desiccation cracks. To counteract these problems rice straw can be applied. We analysed soil microbial responses to different crop rotation systems (rice-rice (RR), maize-maize (MM), rice-maize (MR)) and to rice straw application in the soil and rhizosphere of maize. *Zea mays* was grown in microcosms using soils from different field locations, each including different crop rotation regimes. The bacterial and fungal community composition was analysed by 16S rRNA gene and ITS based amplicon sequencing in the bulk soil and rhizosphere. The microbiota was clearly different in soils from the different field locations (ANOSIM: R = 0.516 for the bacterial community; R = 0.817 for the fungal community). Within the field locations, crop rotation contributed differently to the variation in microbial community composition. Strong differences were observed in communities inhabiting soils under monosuccession (RR vs. MM) (ANOSIM: R = 0.923 for the bacterial and R = 0.714 for the fungal community), while the communities in soils undergoing MR crop rotation were more similar to those of the corresponding RR soils (ANOSIM: R = 0.111 – 0.175). The observed differences could be explained by altered oxygen availabilities in RR and MR soils, resulting in an enrichment of anaerobic bacteria in the soils, and the presence of the different crops, leading to the enrichment of host-plant specific microbial communities. The responses of the microbial communities to the application of rice straw in the microcosms were rather weak compared to the other factors. The taxa responding in bulk soil and rhizosphere were mostly distinct. In conclusion, this study revealed that the different agricultural management practices affect microbial community composition to different extent, not only in the bulk soil but also in the rhizosphere, and that the microbial responses in bulk soil and rhizosphere are distinct.

Introduction

Rice is one of the most important staple foods worldwide and has an increasing demand because of the rising world population (Van Nguyen and Ferrero, 2006). Traditional rice cultivation causes major environmental problems as flooded rice fields represent a major source of atmospheric methane, a greenhouse gas that contributes 20 – 30 % to global warming (Conrad, 2009; Forster et al., 2007). Moreover, rice cultivation under flooded conditions demands two to three times more water than the cultivation of other crops (Tuong et al., 2005). Especially in Asia, farmers need 50 % of the freshwater to irrigate their paddy fields (Tuong et al., 2005). Because of these facts, farmers in tropical and subtropical Asia diversify their cropping systems by introducing a crop rotation system with rice cultivation in the wet season and maize in the dry season (Weller et al., 2015). Maize already dominates the upland agricultural systems in the Philippines, because the requirement of water is much smaller and a simultaneous increasing demand of maize for poultry fattening and biofuel production has been reported (Weller et al., 2015).

The seasonal change between wet and dry conditions in the soil leads to the formation of desiccation cracks during maize cultivation, which cause loss of water, dissolved organic carbon and an increased release of the greenhouse gas N_2O (He et al., 2015; Weller et al., 2015). To prevent crack formation and to stabilize the soil texture, rice straw can be incorporated into the soil at the end of the rice-cropping season. Additionally, surface-application of straw reduces evaporation and thus crack formation (Cabangon and Tuong, 2000). Moreover, straw application is known to improve the physical and biological conditions by preventing soil degradation and increasing soil organic carbon stocks and crop productivity (Chen et al., 2010; Liu et al., 2014).

The conversion of a rice monosuccession system into a rice-maize crop rotation system leads to altered conditions for soil microbial communities; especially the period of anoxic conditions in soil is considerably reduced. The archaeal community composition has been reported to show dramatic changes upon introduction of rice-maize crop rotation. A decrease of anaerobic methanogenic lineages and an increase of aerobic *Thaumarchaeota* was observed in the rotated soil during the rice growing season, whereas the bacterial community was only little affected (Breidenbach et al., 2015). In a different study, Lopes *et al.*, (2014) reported responses of the bacterial community in a paddy soil undergoing crop rotation by introducing alfalfa as upland plant. Crop rotation may specifically affect microorganisms within microbial hot-spot regions of the soil such as the rhizosphere, as plant roots recruit their associated microbiota from the

soil. This has not yet been studied in detail, especially for plants grown in paddy soils under upland conditions.

A modified crop rotation regime will lead to changes in soil carbon supply. All plants release a substantial amount of carbon into the soil via rhizodeposition (Badri and Vivanco, 2009; Bais et al., 2006). Due to the cultivation of maize in a rice cropping system, a different blend of carbon compounds will be released into the soil and a different, plant-host specific microbial community will develop in the rhizosphere (Berg and Smalla, 2009; Edwards et al., 2015; Peiffer et al., 2013). Over time, these changes may contribute to the development of an altered soil microbial community in rice-maize crop rotation systems compared to rice monosuccession systems.

The application of rice straw provides a valuable carbon source for soil microorganisms, and rice straw degradation has been studied in paddy soils under oxic as well as anoxic conditions. Chen *et al.*, (2010) and Conrad *et al.*, (2012) showed that the addition of straw leads to changes in the bacterial and archaeal community composition in paddy soils. An effect was also seen in the rice rhizosphere (Shresta et al 2011). Different bacterial and fungal taxa were identified as part of the straw degrading community (Lee et al., 2011; Murase et al., 2012; Rui et al., 2009; Shrestha et al., 2011). However, the impact of rice straw addition on microbial communities in paddy soils was mostly studied in soils of rice monosuccession systems, while information about the short-term effects of rice straw in crop rotation systems is scarce, where the applied rice straw is mostly degraded under oxic conditions during the period of upland cropping.

The objective of this study was to investigate how agricultural management practices affect soil microbial communities, focussing on the longer-term effects of crop rotation and the short-term responses of straw application. Responses of bacterial and fungal communities were analysed in bulk and rhizosphere soil. We hypothesized that 1.) long-term monosuccession of rice and maize leads to the development of clearly distinct microbial communities in these soils, while the implementation of a rice-maize crop rotation will cause moderate shifts, leading to communities with intermediate appearance. Crop rotation will induce specific microbial responses in the bulk soil and the rhizosphere of the cultivated crop. 2.) The application of rice straw will change microbial community diversity and composition. We expect to see stronger responses in the bulk soil microbial communities than in the rhizosphere microbiota, as the latter is expected to profit predominantly from plant root derived carbon. To address these points, we analysed the composition of bacterial and fungal communities in soils subjected to different crop rotation regimes, i.e. we compared maize monosuccession (MM) or rice-maize crop rotation (MR) to rice monosuccession (RR). Soils were collected from different field sites

and used in microcosm experiments, in which maize was cultivated in the presence or absence of rice straw. Bulk soil samples as well as rhizosphere samples were collected at different time points during the maize growth period.

Material and Methods

Sampling sites

Field locations for soil sample collection were at the International Rice Research Institute (IRRI) in Los Banos, Philippines (14°11'N, 121°15'E), in Tarlac, Philippines (15°32´N, 120°37´E) and Zeme, Pavia, Italy (45°11´N,8°40´E). From each study site, we took soil from two neighbouring fields, which were under different crop rotation regimes for different periods of time (table 1). Each site included a field under rice monosuccession, to which the respective alternative cropping regimes (MR or MM) were compared. Soils with different periods of maize rice crop rotation were taken from IRRI (MR crop rotation since 4 years) and Tarlac (MR crop rotation since 20 years). Soil under maize monosuccession (> 30 years) from Italy was included to compare the impact of MR crop rotation to differences developing under long-term monosuccession regimes. At the Philippine sites, soil samples were taken at the end of the wet season after the rice cultivation period and thus before rice and maize planting. Likewise, the Italian soils were collected in spring before rice/maize planting. All samples were taken from drained soils after plowing. Upon collection, the soil was immediately air-dried and homogenized before the start of the experiment. Basic soil properties including soil type, maximum water holding capacity, pH, nitrogen and carbon content, and the C:N ratio were determined using standard methods (supplementary table 1).

Setup of the microcosm experiment

For the microcosm experiments, the soil was moistened, half of it mixed with chopped rice straw (2 – 5 cm pieces; 6 kg straw m^{-3}), filled into plastic pots (1.2 L pots for sample collection form young plants, 7 L pots for plants older than 21 days), and maize seeds were sown. For bulk soil sampling, pots remained unplanted. The experimental setup included four replicates for all treatments (i.e. for maize with straw, maize without straw, bulk soil with straw and bulk soil without straw) and every time point. The first rhizosphere samples were taken 8 days after sowing, while bulk soil sampling started at the day of sowing; further sampling was performed as listed in table 1. The pots were watered every day and received basal fertilisation of 50 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} at seeding. Nitrogen was applied in three split applications with 30 kg urea ha^{-1} basal at seeding and 50 kg urea ha^{-1} at 29 and 50 days after seeding. The greenhouse experiments with the soils from Italy were conducted in Bonn (Germany), while

the experiments with soils from Tarlac and IRRI were conducted at IRRI, Philippines. For the Italian soils, sampling was performed with high temporal resolution until day 85 to evaluate time-dependent responses in detail, while a lower temporal resolution was chosen for the Philippine samples (Table 1).

Table 1: Experimental setup of the microcosm experiment.

Field location	Crop rotation	Experimental site for microcosm experiment	Maize variety	Time (sample collection time points, days after sowing)
Italy	Rice (RR) since > 30 years	Bonn, Germany	NC358	0, 8, 15, 29, 43, 85
Italy	Maize (MM) since > 30 years	Bonn, Germany	NC358	0, 8, 15, 29, 43, 85
IRRI, Philippines	Rice (RR) since > 50 years	IRRI, Philippines	Pioneer 30T80	0, 15, 43
IRRI, Philippines	Rice-maize (MR) since 4 years	IRRI, Philippines	Pioneer 30T80	0, 15, 43
Tarlac, Philippines	Rice (RR) since 1 year	IRRI, Philippines	Pioneer 30T80	0, 15, 43
Tarlac, Philippines	Rice-maize (MR) since > 20 years	IRRI, Philippines	Pioneer 30T80	0, 15, 43

Rhizosphere and bulk soil sample collection

About 10 g of bulk soil were taken with a sterile spatula from unplanted pots after mixing the soil in the pot and immediately frozen at -20 °C. For rhizosphere sampling, plants were removed from the pots and hand shaken to remove large soil aggregates and loosely adhering soil. The soil remaining attached on the roots was considered to be rhizosphere soil and was collected using a modified protocol of Lundberg *et al.* (2012). Roots with associated rhizosphere soil were placed into a sterile 50 mL tube and submerged with 25 mL 1x phosphate buffered saline (PBS; 1.36 M NaCl, 100 mM Na_2HPO_4, 20 mM KCl, 17 mM KH_2PO_4, 0.02 % Silwet L-77, pH 7.4). Larger samples, collected from plants older than 28 days, were transferred into a sterile 720 mL glass and filled with 300 mL 1x PBS. Thirty or fifty grams (for the larger samples) of sterile glass beads (Ø 4 mm) were added and the samples shaken at 420 rpm for 20 min. The resulting turbid solution was filtered through a 500 µm nylon mesh into new 50 mL tubes to

separate soil and roots. The filtrate was centrifuged for 20 min at 3200 x g. The supernatant was removed and the pellet stored at -20°C until further processing.

Nucleic acid extraction and amplicon sequencing

Soil DNA extraction was performed using the NucleoSpin Soil Kit (Macherey Nagel, Düren, Germany) following the manufacturer's instructions with the following modifications. Microbial cells in 0.3 g of soil were mechanically disrupted by beat beating (TissueLyser, Qiagen, Germany) in the presence of SL1 buffer solution and enhancer solution. The final resuspension of DNA was done in 30 µL PCR-grade water.

16S rRNA genes were amplified using the primer set 515F-806R, targeting a 291 bp product of the V4-V5 region of the 16S rRNA gene from *Bacteria* and *Archaea* (Bates et al., 2011). The fungal ITS1 region was amplified using the primer set ITS1F-ITS2 (Gardes and Bruns, 1993; White et al., 1990), resulting in a 180 bp product. We used a two-step PCR approach in which conventional PCR primers without barcodes were applied to amplify the target region during 30 cycles in the first step. In the second step, the obtained amplicons served as template in a 5 cycle PCR using sample-specific barcode primers. The forward primer included an 8-bp barcode plus a 3-5 bp stagger sequence to increase sequence variability for the Illumina platform. PCR reactions were carried out in technical triplicates and pooled for sequencing. Each replicate 25-µL assay contained 1x Herculase II reaction buffer, 0.25 U Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, USA), 0.25 mM dNTPs, 0.25 µM of forward and reverse primer, 1 mM $MgCl_2$, 0.8 mg mL^{-1} BSA and 1 µL template DNA. The thermal cycling protocol consisted of an initial denaturation step at 95 °C for 2 min, followed by repeated cycles of denaturation at 95 °C for 20 s, annealing at 52 °C for the 16S rRNA gene and 50 °C for the ITS1 region for 20 s, elongation at 72 °C for 20 s and a final elongation step for 3 min.

The PCR products were quantified using the QuantiFluor dsDNA System (Promega, Madison, USA) on an Infinite 200 Pro plate reader (Tecan, Männedorf, Switzerland) at 490 nm excitation and 530 nm emission wavelength. Afterwards, PCR products were pooled at equimolar concentrations. Pooled PCR products were cleaned using the CleanPCR magnetic bead system (CleanNA; Alphen aan den Rijn, Netherlands) according to manufacturer's instructions. Library preparation and sequencing on an Illumina HiSeq system generated paired-end reads (2x250 bp) and was performed by the Max Planck-Genome-centre Cologne. Read files obtained after the quality filtering step were submitted to the EBI SRA public database under the project accession number PRJEB23682.

DNA sequence analysis

Sequence data were assembled with the USEARCH paired-read assembler (Edgar and Flyvbjerg, 2015) to create consensus sequences with a consensus of at least 90 % and a quality score of Q = 2. Sequences were trimmed to remove the reverse primer and sequences < 200 bp were removed (*cutadapt*) (Martin, 2011). Reverse complementary sequences were identified and turned (*fastx_reverse_complement*). The sequences were demultiplexed according to their barcode sequences using an own written perl script. The forward primer was removed after demultiplexing, because barcode sequences are located in front of the forward primer.

Quality filtering and dereplication were conducted using USEARCH v9 (Edgar, 2013) (USEARCH parameter: remove reads with expected number of base call errors exceeding p = 0.01 and Q = 20). The sequences were binned into operational taxonomic units (OTUs) at a threshold of 97 % similarity (corresponding to genus level resolution) using the UPARSE algorithm (Edgar, 2013). This command included chimera filtering. An additional identification of chimeric sequences was done using the uchime2 algorithm (Edgar, 2016b) on USEARCH 9.0 against a reference database. 16S rRNA gene based OTUs were annotated based on representative sequences according to the RDP 16S rRNA training set v16 (Edgar, 2016a; Maidak et al., 2000), while ITS OTUs were taxonomically identified according to the UNITE ITS database (version 7.1; Abarenkov et al., 2010). For taxonomy prediction, a cut-off value of 0.8 was chosen. Finally, 16S rRNA gene sequences identified as chloroplasts were removed from the 16S rRNA gene sequence dataset (0.5 % of the sequences).

Statistical analysis

Statistical analyses were conducted in STAMP (Parks and Beiko, 2010) and in R using the packages Vegan (Oksanen et al., 2016) and Phyloseq (McMurdie and Holmes, 2013). For all OTU based analyses, the original OTU table was filtered to contain only sequences that were taxonomically classified as *Fungi* or *Bacteria* and *Archaea* and OTUs represented by a maximum of only two reads in one or more samples were discarded. Estimation of Alpha-diversity was based on an evenly rarefied OTU table and included calculation of the observed richness via Chao1. To test for significant differences in bacterial and fungal Alpha-diversity between groups of samples, non-parametric Kruskal-Wallis tests were performed, as Shapiro-Wilk test revealed non-normal data distribution.

The structure of the microbial communities was evaluated at high taxonomic resolution (97% sequence identity) and ordinated in non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity matrices. For NMDS ordination, the OTU tables were pre-

processed, so that sparse OTUs were removed. The sparsity threshold was 0.5, meaning that an OTU not found in at least 50 % of the samples was removed as statistically uninformative. To test for significant differences between groups of samples, an analysis of similarity (ANOSIM) was performed in Vegan with 999 permutations based on Bray-Curtis distances between samples. In case of multiple comparisons, *P*-values were Bonferroni-Holm corrected. NMDS and ANOSIM were performed on successively reduced datasets, beginning with an overall analysis (including all locations, compartments, time points and straw treatments). This was followed by the analysis of subsampled datasets to assess the impact of crop rotation and straw treatment in more detail. Crop rotation was evaluated i) within each field location (including compartments, time points and straw treatments)), ii) within each compartment (including time points and straw treatments), iii) at different time points (including straw treatments). For straw treatment, the data were completely dissected. This procedure followed the succession from the most significant to the least significant impact factor, enabling to evaluate the effect of each treatment more specifically and to exclude effects of co-variants.

The impact of different treatments on individual taxa was analysed using the program STAMP (Parks and Beiko, 2010). First, genera were identified that responded to crop rotation within each field location. The datasets were then further subsampled by compartment to compare crop rotation responsive taxa between bulk soil and rhizosphere. To identify genera that were significantly impacted by straw addition, the datasets were subsampled according to field location, crop rotation and compartment. Significant differences between groups of samples were tested with Kruskal-Wallis H test and multiple comparison corrections were done with Benjamini-Hochberg FDR.

Results

Identification of major factors affecting diversity and composition of bacterial and fungal communities

Bacterial and fungal communities were analysed in soil and rhizosphere samples by 16S rRNA gene and ITS1 amplicon sequencing, respectively. After pre-processing and quality filtering, about 16,500 reads per sample of 16S rRNA gene sequences and 13,900 reads per sample of the ITS1 region remained. In a first instance, the data of all samples were combined to identify major factors that influenced microbial diversity and community composition. The effects of crop rotation, straw treatment, field location and compartment as well as variation due to incubation time were evaluated. A comparison of OTU richness and Chao 1 diversity indices revealed that all factors had a significant influence on bacterial and fungal diversity (supplementary table 2). The diversity was most strongly influenced by crop rotation and least by straw application (in case of bacteria) and compartment (in case of fungi).

In all soils the bacterial communities were dominated by the phyla *Acidobacteria*, *Chloroflexi* and *Proteobacteria* (classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria)*, while fungal communities consisted mainly of members of the classes *Sordariomycetes*, *Dithideomycetes*, *Eurotiomycetes* and *Agaricomycetes* (supplementary figure 1). Differences in the composition of the microbial communities between all samples based on NMDS plots in combination with ANOSIM revealed that field location and crop rotation were the most relevant factors explaining dissimilarities between samples (figure 1). ANOSIM showed that field location affected in particular the fungal communities, as evident from the high R-value of 0.817 ($P < 0.001$), while variation in bacterial communities due to field location resulted in an intermediate R-value of 0.516 ($P < 0.001$). The effect of crop rotation on bacterial communities was also intermediate ($R = 0.545$; $P < 0.001$), while it was lower for fungal communities ($R = 0.359$; $P < 0.001$). The factors compartment, time and straw had a weaker effect on the overall microbial community composition. These findings were confirmed by a cluster analysis, performed at class level resolution (supplementary figure 2). Because of the strong differences due to field location, the samples from each location were analysed separately to assess the impact of crop rotation and straw application in more detail.

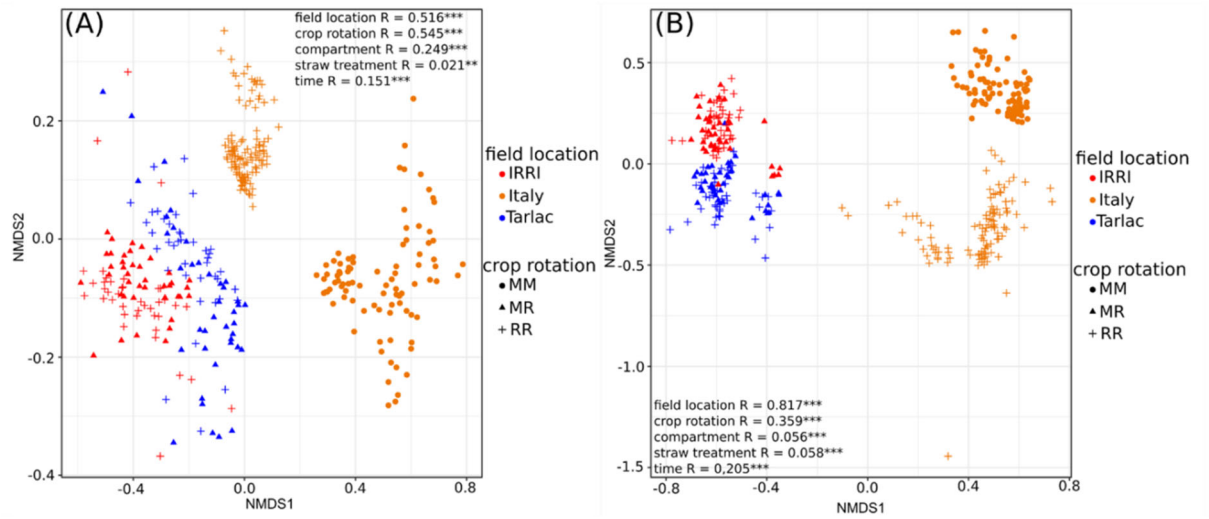


Figure 1: Ordination plots showing the influence of field location and crop rotation on bacterial (A) and fungal (B) community composition. NMDS plots based on Bray-Curtis dissimilarities were calculated from relative OTU abundances. Results of ANOSIM are shown with P < 0.05 *, P < 0.01**, P < 0.001*** for all grouping factors.

Impact of crop rotation on microbial diversity and community composition within field locations

Strongest differences in diversity in response to crop rotation were observed in the Italian soil samples (figure 2), where the diversity of bacteria and fungi was 1.3-fold to 1.5-fold higher in RR soil than in MM soil ($P < 0.001$). This was observed in the bulk soil as well as in the rhizosphere. In IRRI and Tarlac soils, crop rotation affected microbial diversity less consistently. The fungal diversity was modulated by crop rotation in the rhizosphere of IRRI soil and the bacterial diversity in the bulk soil of Tarlac ($P < 0.05$).

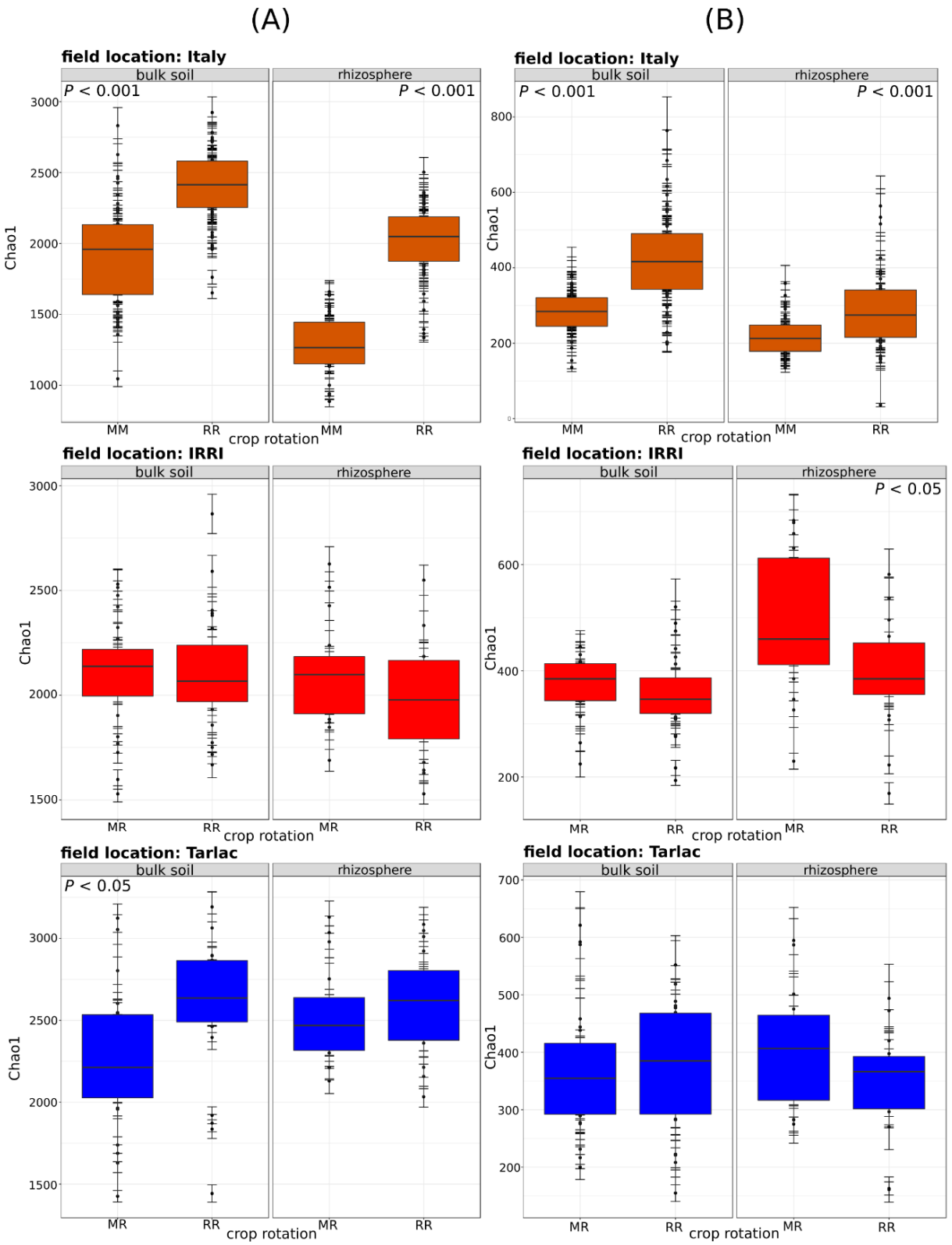


Figure 2: Box plots showing the influence of crop rotation on bacterial (column A) and fungal alpha diversity (column B) within each field location and compartment. Each box plot shows alpha diversity based on the Chao1 diversity index and includes all samples treated with or without straw and collected at different time points. The median as well as the 25th and 75th percentile of the samples is presented, individual data points outside of this range are given as mean ± standard deviation of four replicate samples. Significant differences due to crop rotations within one compartment (bulk soil or rhizosphere) are noted.

Field location specific NMDS ordinations along with ANOSIM (figure 3) revealed that crop rotation had a very strong impact on the bacterial (R = 0.923; $P < 0.001$) and fungal (R = 0.714; $P < 0.001$) community composition in the Italian soils, where rice and maize were grown in monosuccession for > 30 years, respectively. A further clear structuring of the communities in these soils was evident according to compartment (R = 0.302 for bacteria, R = 0.258 for fungi; both $P < 0.001$), while the factors time and straw application explained less variation. In the Philippine soils, which were managed under RR or MR, crop rotation explained less of the variation in microbial community composition (R-values between 0.111 and 0.175) and was in most cases less important compared to the impact of compartment and time (R-values between 0.112 and 0.361). As the impact of crop rotation was covered by compartment and time in these soils, it was evaluated more specifically by ANOSIM within each compartment and at each time point (supplementary table 3). This revealed a significant response of the microbial community to crop rotation in all individual cases. The response remained strongest for the soils from Italy and was of equal strength in the soils from IRRI and Tarlac. Moreover, crop rotation had as stronger effect on the bacterial than the fungal community ($P < 0.005$). Responses in the bulk soil and rhizosphere were of comparable strength, and a trend over time was not evident.

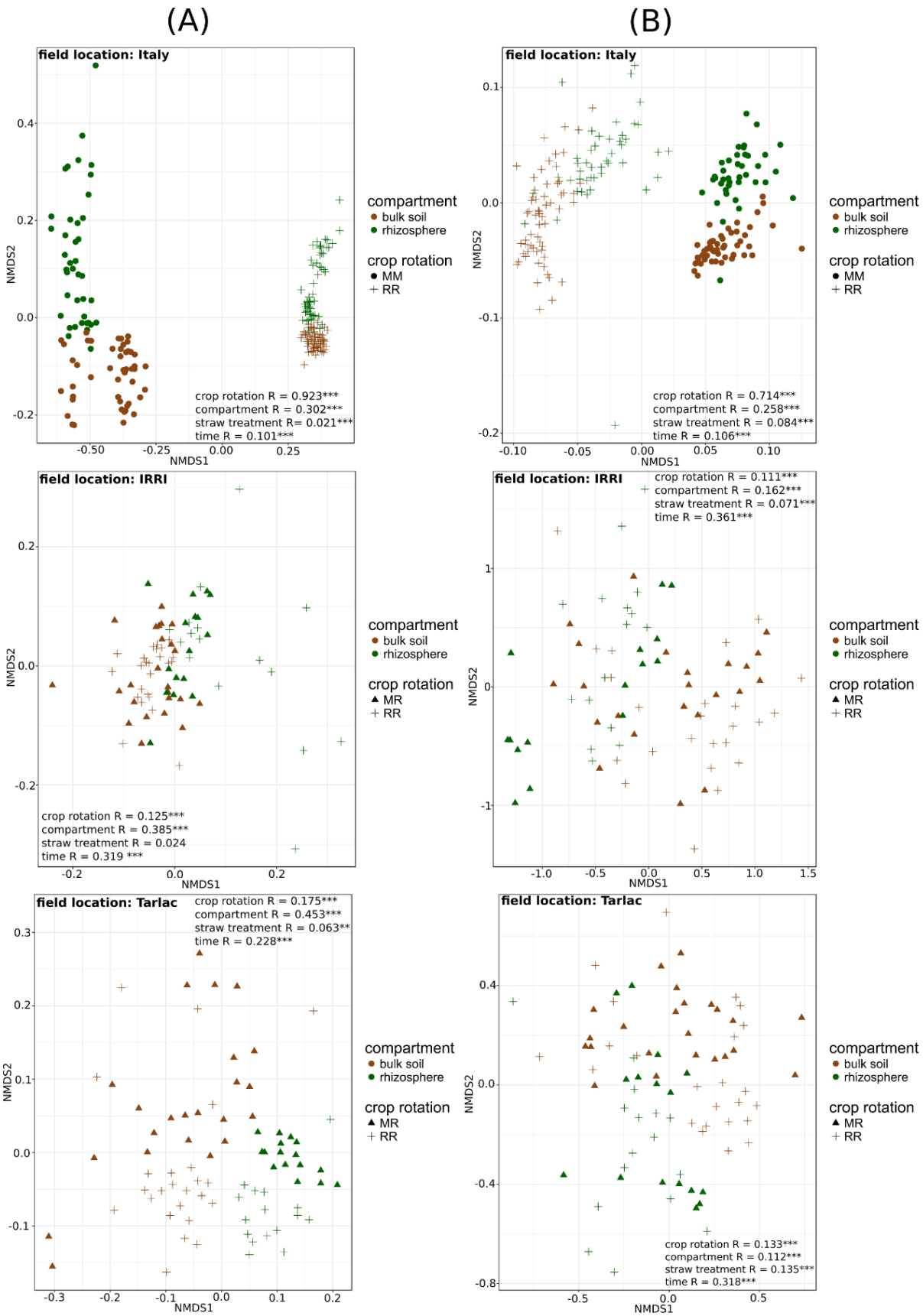


Figure 3: NMDS plots of 16S rRNA (column A) and ITS1 (column B) community composition in soils from different field locations. NMDS plots based on Bray-Curtis similarities were calculated from relative OTU abundances. ANOSIM was applied to test for differences in community composition due to compartment, crop rotation, time and straw treatment. R-values are shown with $P < 0.05$*, $P < 0.01$**, $P < 0.001$***.

We performed analyses in STAMP to identify bacterial and fungal genera that responded to crop rotation within each field location. In agreement with the clear impact of crop rotation at the Italian site, the highest number of responsive genera (361 bacterial and 94 fungal) was observed here (listed in supplementary tables 6 A, B), while the numbers were much lower in soils from IRRI and Tarlac (25 and 41 bacterial genera, 4 and 5 fungal genera). More than half of the genera that were identified as responsive in a Philippine soil showed a similar response in soils from Italy. Most responses were observed within the phyla *Actinobacteria, Acidobacteria, Firmicutes* and *Proteobacteria*. Genera of the classes *Actinobacteria, Alphaproteobacteria*, *Gammaproteobacteria* and *Bacilli* were predominantly enriched in the Italian MM soil (figure 4). Likewise, genera of *Actinobacteria*, *Bacilli*, and *Gammaproteobacteria* were enriched in the Philippine MR soils. In contrast, genera of *Deltaproteobacteria,* including sulfate and iron reducers, were consistently enriched in all RR soils. Moreover, diverse genera of *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes* and the class *Alphaproteobacteria* were specifically enriched in some of the RR soils. In the fungal communities, most differences were observed within the phyla *Ascomycota* and *Basidiomycota.* Especially genera of the class *Sordariomycetes* and *Agaricomycetes* were enriched in the Italian RR or MM soil (figure 4).

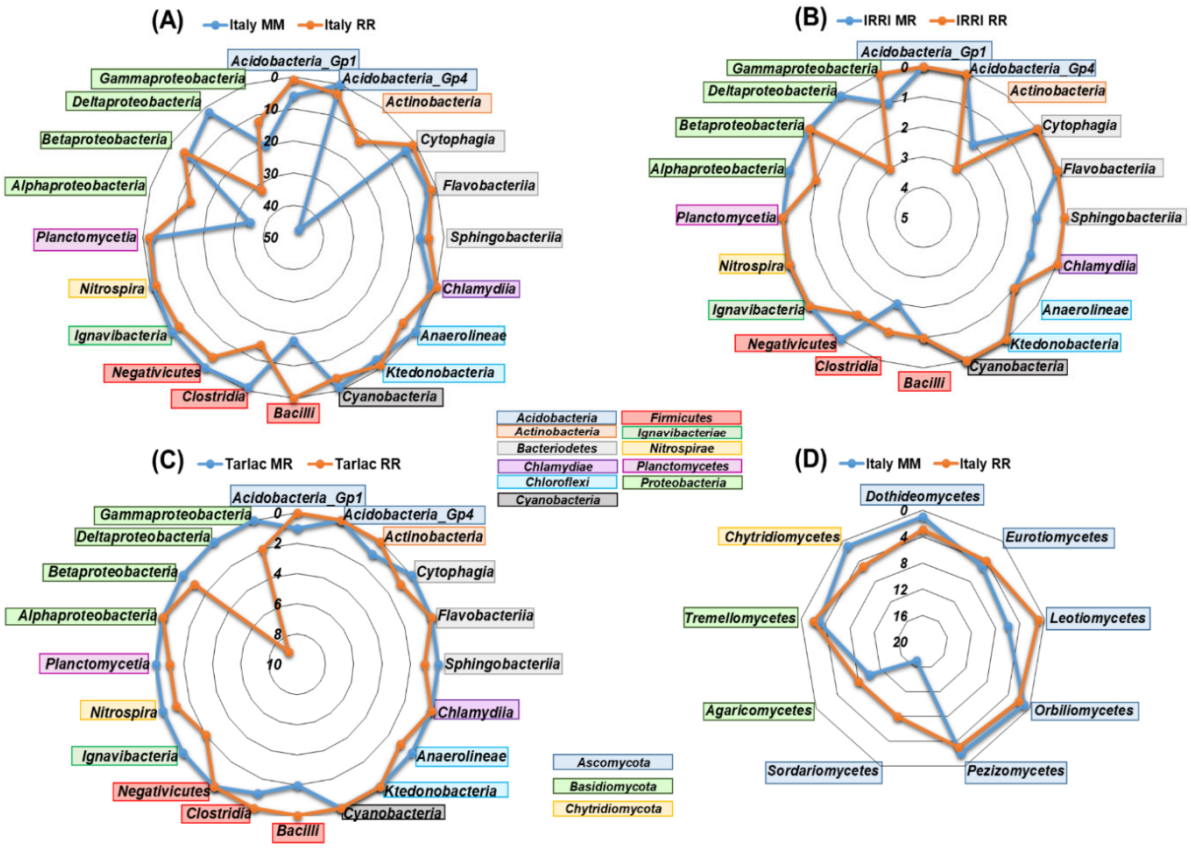


Figure 4: Radar charts showing the number of bacterial (A), (B), (C) and fungal (D) genera in different classes that were identified as significantly enriched by crop rotation in the different field locations (Italy, IRRI, Tarlac) based on STAMP analysis. Displayed are classes for which at least three different genera were identified as specifically enriched in one or the other soil. Fungal responses to crop rotation at IRRI and Tarlac were weak and are therefore not included in the figure.

Because compartment-specific responses to crop rotation were of particular interest, an additional analysis in STAMP was done with datasets separated further by compartment. Overall, the analysis resulted in a comparable number of responsive genera in MM, MR and RR soils at the respective field locations (listed in supplementary tables 6 C, D). Thus, the further dissection of the datasets according to compartment did not result in the identification of a higher number of responsive taxa in the Philippine soils, where compartment masked the effect of crop rotation to some extent. As before, differences were most evident in soils from Italy and more differences were observed for bacterial than fungal communities. Focussing on compartment-specific responses to crop rotation in the Italian soils, the analysis revealed a higher number of responsive bacterial genera in the bulk soil (165 specific for MM and 176 for RR) than in the rhizosphere (143 specific for MM and 126 for RR). The percentage of bacterial genera that responded in both compartments was high, with 45 % and 60 % for MM and RR soils, respectively (supplementary figure 3). The same trends were observed in most Philippine soils. Fungal communities showed less overlap (maximum 25%) between compartments. Among the genera that were enriched in the Italian MM soils, members of *Bacilli* and

Gammaproteobacteria were more specifically found in the bulk soil, while *Betaproteobacteria* were more specifically responding to crop rotation in the rhizosphere (supplementary figure 4). In the Italian RR soil, genera of *Actinobacteria*, *Clostridia*, *Alphaproteobacteria* and *Deltaproteobacteria* were more responsive to crop rotation in the bulk soil, likewise as the fungal genera of the class *Sordariomycetes*.

Impact of straw application on microbial communities

In comparison to the other factors, straw application had the weakest impact on the bacterial and fungal diversity (supplementary table 2) and community composition (figure 3). ANOSIM revealed that a straw effect was most evident in the fungal community in Tarlac soils ($R = 0.135$, $P < 0.001$), but was hardly detectable in the bacterial communities. To evaluate the effect of straw application more specifically, the datasets were completely dissected so that samples representing one field location, one type of crop rotation and one compartment were analysed individually per time point. This revealed a straw effect in the majority of cases (approx. 70% of all datasets) according to ANOSIM (supplementary table 4). More significant and higher R-values were observed for fungal than bacterial communities, indicating a stronger response of fungal communities to straw application (figure 5). Remarkably, responses to straw were stronger in the rhizosphere than in bulk soil. This was also seen when applying ANOSIM to less dissected datasets (supplementary table 5). A clear trend over time concerning the responses of bacterial and fungal communities to straw application was not evident.

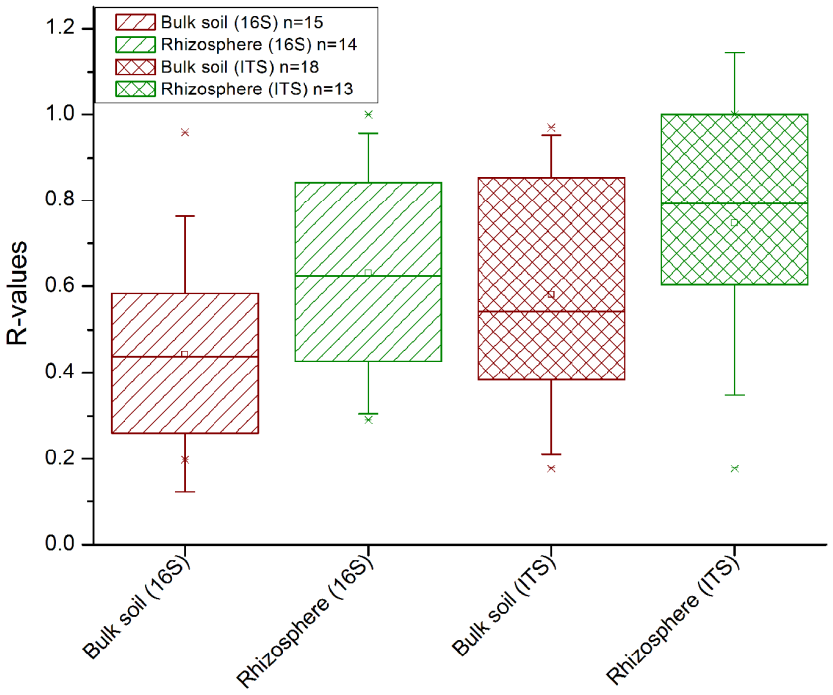


Figure 5: Influence of straw treatment on the bacterial (16S) and fungal (ITS1) community composition in the rhizosphere and bulk soil. Data from each field, each compartment and of every time point were analyzed separately by ANOSIM. All significant R-values from this analysis (as shown in supplementary table 4) are included in this plot. Between 62 and 78 % of the R-values were significant ($P < 0.05$) per group displayed.

To identify bacterial and fungal genera that showed a significant increase in relative abundance due to straw application, we performed an analysis in STAMP. Samples with straw application were compared to those without straw application, resolved for each field location, crop rotation regime and compartment (full list of genera in supplementary tables 6 E, F). A specific enrichment of bacterial and fungal genera due to straw application was predominantly observed in the Italian soils and in the rhizosphere of Tarlac MR soil. Between 5 and 32 different bacterial genera and 2 to 4 fungal genera were identified per compartment (Table 2). More than 80% of these genera were either specifically detected in the rhizosphere or in bulk soil, but not in both compartments of a soil. Most consistently detected across compartments and field locations were members in the classes *Alphaproteobacteria*, *Actinobacteria* and *Verrucomicrobiae*. In the fungal communities, genera belonging to the *Sordariomycetes* responded most consistently. Remarkably, several fungal taxa showed a significantly higher relative abundance in rhizosphere and bulk soil samples without straw application, including members of the *Eurotiomycetes, Microbotryomycetes, Leotiomycetes, Pezizomycetes* and *Sordariomycetes* (supplementary table 6 F).

Table 2: Number of genera significantly enriched in soils with straw application.

Phylum	Class	Italy MM		Italy RR		Tarlac MR
		Bulk soil	Rhizo-sphere	Bulk soil	Rhizo-sphere	Rhizo-sphere
Acidobacteria	Acidobacteria Gp1	1	1			
	Acidobacteria Gp4				1	1
	Acidobacteria Gp6				1	
	Acidobacteria Gp16					1
Actinobacteria	Actinobacteria	4		3	4	4
Armatimonadetes	Armatimonadetes gp4				1	
	Fimbriimonadia				1	
Bacteroidetes	Cytophagia			3		1
	Flavobacteriia				1	
	Sphingobacteriia	2		3	1	
	Unclassified Bacteroidetes			1		
Candidate division WPS-1	WPS-1, genera incertae sedis				1	
Firmicutes	Bacilli	1		2	1	
Planctomycetes	Planctomycetia		1	1	1	
Proteobacteria	Alphaproteobacteria	5	3	11	8	7
	Betaproteobacteria	1			2	1
	Deltaproteobacteria			3		
	Gammaproteobacteria	1		3	2	
Verrucomicrobia	Opitutae				1	
	Spartobacteria			1		
	Verrucomicrobiae	2		1	4	1
Total number of enriched bacterial genera		17	5	32	30	16
Ascomycota	Dothideomycetes	1				
	Eurotiomycetes	1			1	
	Sordariomycetes	2	2	1	2	
Chytridiomycota	Chytridiomycetes		1			
Zygomycota	Mucoromycotina, class incertae sedis			1		
Total number of enriched fungal genera		4	3	2	3	

Discussion

Relevance of crop rotation and straw application on soil microbial communities in relation to field location, compartment and time

The effect of two agricultural management practices on soil microbial communities was evaluated, i. e. responses due to the introduction of a crop rotation regime in RR soils and short-term responses to rice straw application. We assessed the effects of these management practices in relation to the impact of field location, compartment and over time, as these factors are known to affect microbial communities in the bulk soil and rhizosphere (Castellanos et al., 2009; Lee et al., 2011; Peiffer et al., 2013). Our data revealed that diversity as well as community composition were significantly influenced by all these factors. Overall, community composition was most different in samples from different field locations and least affected by straw application. The influence of crop rotation on soil microbial community composition was intermediate compared to the impact of the other factors.

The finding that field location had the strongest impact and that soils from Italy harboured the most distinct microbial communities is in good agreement with the fact that fields in different geographic regions and climatic zones are known to contribute to differences in microbial community composition (Brockett et al., 2012; Castellanos et al., 2009; Neufeld and Mohn, 2005; Peiffer et al., 2013). Differences may also be due to variation in soil physicochemical characteristics. Variation was observed with regard to soil type, clay content, organic carbon content, pH and water holding capacity (supplementary table 1), factors that are known to influence soil microbial community composition (Bai et al., 2017; Brockett et al., 2012; Zhao et al., 2014, 2016). Besides, the experimental setup has possibly contributed to the observed differences between Italian and Philippine soils to some extent. The experiment with Italian soil was performed in a different greenhouse and with a different maize variety compared to the Philippine soils (table 1). To assess the possible impact related to this difference, a control experiment with RR soil from IRRI was included in the microcosm experiment that was performed with the Italian soils. It revealed that the methodological difference was not the major factor for the observed differences between Italian and Philippine soils, because this IRRI soil remained clearly different from the Italian soils and similar, though not identical, to the other IRRI soils (supplementary figure 5).

Besides field location, compartment contributed substantially to the overall differences in soil microbial community composition and diversity, though the impact was weaker compared to field location (figure 3), likewise as observed in previous studies (Castellanos et al., 2009;

Peiffer et al., 2013). Moreover, differences in community composition between rhizosphere and bulk soil were smaller for fungal communities than for bacterial communities, as reported earlier (Granzow et al., 2017; Pausch et al., 2016; Uroz et al., 2016). The factor time also caused variation in fungal and bacterial community composition and diversity (figure 3, supplementary table 2). The variation over time was lower in bulk soil than in the rhizosphere (supplementary table 5). This was expected, as microbial communities are known to develop in the rhizosphere over time along with the plant (Qiao et al., 2017; Smalla et al., 2001; Wang et al., 2016). Changes in the bulk soil can be explained by the straw degradation process, which induces successional changes in microbial community composition during residue decomposition (Bastian et al., 2009; Conrad et al., 2012; Tardy et al., 2015). This process may also have contributed to the time-dependent changes observed in the rhizosphere.

Impact of crop rotation on microbial community composition and diversity

The effect of crop rotation on microbial community composition and diversity was evident in soils from all three field locations, but strongest in the Italian soils, where rice and maize were cultivated in monosuccession in adjacently located fields for more than 30 years (figure 3, supplementary table 2). In the Philippine soils, where the impact of MR crop rotation was evaluated in comparison to RR, changes in the crop rotation regime occurred for shorter periods of time, so that microbial communities may not yet have fully adapted to the altered conditions. Nevertheless, effects of crop rotation on microbial community composition were evident (figure 3). Responses to crop rotation were reported in previous studies, in which the soil bacterial and fungal community composition was analysed in paddy soils under different crop rotation regimes such as winter wheat – rice or alfalfa – rice, (Lopes et al., 2014; Zhao et al., 2014). Moreover, our findings are in agreement with Breidenbach et al. (2015), who showed that the introduction of a MR crop rotation practice does not change the structure of the bacterial community drastically within the first three years after introducing a MR crop rotation regime.

Two major factors may have contributed to the crop rotation dependent differences, the regular flooding of the fields, leading to periodically anoxic conditions, and the influence of crops that were repeatedly cultured in the soil. Host plant specific rhizosphere communities are known to develop due to plant species specific rhizodeposition processes (Berg and Smalla, 2009; Ladygina and Hedlund, 2010). The long-term release of plant species specific carbon compounds under maize versus rice monosuccession has probably supported the enrichment of a specific microbial community in soil. Moreover, the soil microbiota may have been affected by crop rotation specific management practices such as fertilisation regimes, plant residue input or pest control treatments (Dick, 1992; Geisseler and Scow, 2014; Hussain et al., 2009; Thiele-

Bruhn et al., 2012). In particular the regular change between oxic and anoxic conditions in RR and MR soils vs. MM soils, which limits oxygen availability in soil, has to be considered, because oxygen availability is a well-known factor shaping microbial community composition (Kikuchi et al., 2007; Noll et al., 2005; Zhao et al., 2014). The relevance of this factor is confirmed by the finding that diverse facultative and obligate anaerobic microorganisms were enriched in all RR soils and were not in all cases strongly depleted in MR soils (figure 4). When comparing the differences in microbial community composition between soils under different crop rotation regimes, it is obvious that MR soils are still largely similar to RR soils, while MM soils are clearly distinct from the corresponding RR soil (figure 1). The introduction of flooding periods in an upland soil has obviously a much stronger impact on the microbial community composition than the extension or reduction of recurring flooding periods, as it occuring in RR and MR soils. Other studies also suggest that periodically anoxic conditions in MR crop rotation systems help to maintain a community structure similar to those in RR soils (Breidenbach and Conrad, 2015; Zhao et al., 2014).

Microbial taxa responding to crop rotation

In response to crop rotation, the highest numbers of bacterial and fungal genera were identified in the Italian soils, which is in agreement with the strong differences observed in NMDS plots and ANOSIM analyses for these soils (figure 3). In the RR soils, diverse facultative and obligate anaerobic bacterial genera were significantly enriched (figure 4, supplementary tables 6 A, B), many of them well-known as colonizers in rice field soils (Ahn et al., 2012; Edwards et al., 2015; Itoh et al., 2013; Knief et al., 2012; Lopes et al., 2014; Lu et al., 2006). These include members of the phylum *Verrucomicrobia* (*Prosthecobacter, Opitutus*) as well as members of the classes *Anaerolineae, Ignavibacteria, Negativicutes* and *Clostridia*. Moreover, several genera of deltaproteobacterial sulphate and iron reducers (*Geobacter, Anaeromyxobacter*), methanotrophic bacteria (*Methylocaldum*, *Methylomonas*) and methanogenic archaea (*Methanobacterium*, *Methanomassiliicoccus*) were specifically enriched in RR soils.

In MM and MR soils, several genera belonging to the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacilli* and *Actinobacteria* were specifically enriched (figure 4). These are commonly detected in soil, including maize field soils (Benitez et al., 2017; Li et al., 2014; Zhao et al., 2016) or crop rotation systems with maize (Zhao et al., 2014). Interestingly, the nitrifying bacterial genera *Nitrospira* and *Nitrosococcus* were enriched in the Italian RR soil, while the archaeal genus *Nitrososphaera* was enriched in the corresponding MM soil. Thus, a switch from bacterial to archaeal nitrification appears to be linked to RR versus MM monosuccession. Similarly, Breidenbach et al., (2015) observed that members of the genus

Nitrososphaera were enriched in a MR soil compared to RR soil. However, in some other studies, ammonium oxidizing archaea were found to be more abundant and active under the oxygen-limiting conditions in rice field soils (Azziz et al., 2016; Ke et al., 2015; Wang et al., 2014, 2015, 2017).

The number of fungal genera that were influenced by crop rotation was lower, which corresponds to the lower richness in the fungal communities. Among the fungi enriched in the Italian MM soil were *Leotiomycetes* and *Glomeromycetes*. Members of *Leotiomycetes* are known as maize endophytes (Wang et al., 2006) and *Glomeromycetes* are well-known as arbuscular mycorrhizal symbionts of maize, including the enriched genus *Entrophospora* (Colombo et al., 2017; Na Bhadalung et al., 2005). Actually, the genus *Entrophospora* was enriched in the MM bulk soil rather than in the rhizosphere (supplementary table 6 B). This fungus obviously did not undergo a symbiotic interaction with maize in our microcosm experiment, possibly because we applied fertiliser to provide sufficient nutrients for plant growth (Na Bhadalung et al., 2005). Moreover, *Piriformospora* was significantly enriched in MM bulk soil and the RR rhizosphere soil. The specific enrichment in RR rhizosphere soil rather than in the maize rhizosphere is surprising, as this fungus is better known for its association with maize (Qiang et al., 2012). Further genera known as plant endophytes were enriched in RR or MM soil, including *Pyrenochaetopsis, Exophiala, Penicillium, Paecilomyces* and *Preussia* (Bilal et al., 2017; Papizadeh et al., 2017). Besides the enrichment of beneficial fungi, a maize pathogen, *Ustilago*, was found in the maize rhizosphere in MM soil (Brefort et al., 2009). Taken together, these findings demonstrate very well that crop monosuccession regimes lead to the enrichment of host-plant specific beneficial as well as pathogenic microorganisms.

Members of the *Dothideomycetes* and *Chytridiomycetes* were more specifically detected in the Italian RR soil than in MM soil. The *Dothideomycetes* are a diverse class of fungi, including saprobic and aquatic organisms (Hyde et al., 2013). Similarly, the RR enriched genera *Delfinachytrium, Aquamyces, Betamyces* and *Udeniomyces*, representing *Chytridiomycetes* and *Tremellomycetes*, are usually known from aquatic habitats (Brizzio et al., 2007; Letcher et al., 2008; Vélez et al., 2013; Zhang et al., 2016). These genera are obviously capable to establish populations in paddy rice ecosystems. Several further genera that were significantly enriched in RR or MM soils and are involved in the degradation of organic material, e. g. *Thermomyces*, *Chaetosphaeria, Mrakia* or *Udeniomyeces* (Brizzio et al., 2007; Reblova and Winka, 2000; Zhang et al., 2015). In conclusion, these findings suggest that the specific enrichment of fungal taxa in MM or RR soils is partly driven by the flooding regime during rice cultivation, leading

to the enrichment of fungi that are known from aquatic environments, as well as by the plant, leading to the enrichment of plant-host specific symbionts and pathogens. Moreover, saprotrophic fungi are affected, probably by the supply of organic carbon compounds, which differ to some extent in dependence on the cultivated crop.

Compartment-specific responses to crop rotation

Differences in response to crop rotation were not only evident in the bulk soil, but seen to a similar extent in the maize rhizosphere according to ANOSIM results. Even over time, i.e. up to 43 or 85 days of plant development, the maize rhizosphere microbiota remained clearly distinct in the soils under different crop rotation regimes (supplementary table 3). This demonstrates that crop rotation regimes do not only affect bulk soil microbial communities, but also those in the plant rhizosphere. The compartment-specific analysis in STAMP revealed that more genera responding to crop rotation were identified in the bulk soil than in the rhizosphere (supplementary figure 3). However, the rhizosphere microbiota is less diverse compared to bulk soil (Peiffer et al., 2013), resulting in a lower number of potentially responsive taxa. A very clear response of the rhizosphere microbiota was not necessarily expected, because these microorganisms are largely controlled by plant root released carbon, which is known to shape the rhizosphere microbiota (Berg and Smalla, 2009, Bulgarelli et al., 2013). The high overlap of responsive genera in the bulk soil and rhizosphere (supplementary figure 3) indicates that part of the rhizosphere response is identical to that in bulk soil. This may to some extent be attributed to bulk soil organisms residing in the rhizosphere. Bacterial taxa occurring in bulk soil probably inhabit to some extent the rhizosphere without being part of a very specific plant-supported rhizosphere microbiota, especially when considering that the transition from rhizosphere to bulk soil is continuous. With increasing distance from the plant root surface, the number of plant-supported microorganisms will gradually decrease and bulk soil microorganisms will increase in relative abundance.

Impact of straw application on microbial community composition and diversity in bulk soil and rhizosphere

The short-term responses to straw application were rather weak compared to the other factors in soils from all three field locations (figures 1 and 3). They became evident only after excluding the variation caused by field location, compartment, crop rotation (supplementary table 5) and time (supplementary table 4). In contrast to our results, Tardy et al. (2015) observed that straw application had a stronger impact than crop rotation when comparing grassland with cropland soil. The higher impact of crop rotation in our study can in case of the Italian soils be explained

by the highly different cropping conditions for rice versus maize, which induced substantial changes in the microbial communities. At the Philippine sites, the strength of the impact of crop rotation and straw application was roughly equal, at least for the fungal communities, largely due to the lower impact of crop rotation at these sites (figure 3). After the complete dissection of the datasets, the response of the microbial community to straw application became more evident (supplementary table 4).

Straw application affected the fungal communities more strongly than the bacterial communities (figure 5, supplementary table 5). Saprotrophic fungi are known as effective decomposers contributing to the decomposition of organic matter and thus boost carbon mineralisation in soil (Crowther et al., 2012; Dini-Andreote et al., 2016; Kjøller and Struwe, 2002). Moreover, fungi have been reported to be the dominant group involved in rice straw degradation in RR soils under oxic conditions (Nakamura et al., 2003). We observed a straw-dependent enrichment of genera in the classes *Sordariomyces* and *Dothideomycetes* (family *Sporormiaceae*) in the Italian soils. These are known to play a role in the degradation of plant residue (Phukhamsakda et al., 2016; Tardy et al., 2015; Zhao et al., 2013). Remarkably, the number of fungal genera that were identified as significantly enriched was higher in treatments without straw than in treatments with straw (supplementary table 6 F). This was observed in the rhizosphere as well as in bulk soil. Selective grazing may have affected the fungal populations in straw-supplemented soils due to the presence of high amounts of organic substrate and thus higher overall biological activity, possibly leading to a decrease in relative abundance of fungal taxa in the straw-supplemented soils. To elucidate this phenomenon in more detail, absolute abundances of selected taxa would have to be evaluated and ^{13}C-straw labelling experiments could be performed to study the flow of carbon into the microbial food web in more detail.

In the bacterial community, straw application resulted in an enrichment of diverse bacterial genera (Table 2). Several of them are known as straw or plant residue degrading organisms, including members of *Verrucomicrobiae, Actinobacteria, Bacteroidetes* or the different classes of *Proteobacteria* (Bernard et al., 2007; Fan et al., 2014; Pascault et al., 2013; Semenov et al., 2012). The bacterial genera that were enriched by straw in the rhizosphere belong to phyla and classes that are well known to colonize the (maize) rhizosphere (Bulgarelli et al., 2013; Da Rocha et al., 2013; Hernández et al., 2015; Peiffer et al., 2013). Most genera were detected in either the rhizosphere or the bulk soil of a soil, but not in both compartments. This compartment specific response indicates that rhizosphere-inhabiting microorganisms may profit from plant-derived carbon as well as carbon available from straw application. This was proposed earlier

by Shrestha et al. (2011), who studied the assimilation of rice straw in the rhizosphere of rice plants.

ANOSIM results suggested that the application of straw can induce stronger changes in the rhizosphere microbial communities than in bulk soil communities (Figure 5, supplementary table 5). STAMP analysis confirmed this in case of the Tarlac MR soil by identifying a higher number of genera being significantly enriched in the rhizosphere (Table 2). A stronger response to straw in the rhizosphere would not necessarily be expected, as the rhizosphere microbiota is considered to be mainly influenced by the plant (Berg and Smalla, 2009; Bulgarelli et al., 2013). However, the availability of easy to degrade plant root exudates may have stimulated rice straw degradation in the rhizosphere. These processes may have resulted in stronger shifts in the microbial community composition upon straw application in the rhizosphere. Such rhizosphere priming effects are well known and can improve the plant nutrient status by releasing nutrients upon mineralization of more difficult to degrade organic carbon compounds (Huo et al., 2017).

Conclusions

Field location, followed by crop rotation, incubation time and compartment were identified as main factors influencing microbial community composition and diversity, while the addition of straw had a minor effect. The analysis of the Italian soils revealed that long-term monosuccessionally managed soils developed substantial differences in microbial community composition, which could be well explained by alterations in oxygen availability in soil and the different cultivated crops, leading to the enrichment of plant species-specific microbial mutualists as well as pathogens. Moreover, the plant species-specific carbon supply into the soil most likely influenced the heterotrophic soil microbial community. Soils under MR crop rotation harboured microbial communities that were more alike those in rice soil than in maize soil. Obviously, the anaerobic microbial population is largely maintained in MR soils. Nevertheless, differences between RR and MR were mostly due to a depletion of anaerobic microorganisms in the MR soils. This is in agreement with the expectation that the ecologically more friendly MR crop rotation practice in comparison to rice monosuccession leads to a reduction of greenhouse gas emissions (Weller et al., 2015). The short-term responses to the addition of straw became most evident after exclusion of all other factors assessed in this study. The fungal community responded more strongly than the bacterial community, but in contrast to the bacterial community more taxa of the fungal community were depleted in relative abundance in the presence of straw than enriched. It will be of interest to assess in the future also the longer-term responses of the soil and rhizosphere microbiota to recurring straw applications and microbial carbon cycling in such agricultural systems. Both management

practices, crop rotation and straw application, affected not only the microbial community in the bulk soil, but to roughly similar extent those in the rhizosphere. This indicates that the rhizosphere microbiota is influenced by crop rotation and may not only profit from root-derived carbon. Actually, this influence may increase with decreasing distance to the root, as the transition from the rhizosphere to bulk soil is continuous.

Acknowledgements

We are thankful to the International Rice Research Institute (IRRI), especially Rainer Wassmann, for providing research space. We thank Daniel Said-Pullicino and Marco Romani (Dept. Agricultural, Forest and Food Sciences University of Turin) for providing the soil samples from Italy and Frank Hochholdinger (INRES, University of Bonn) for providing maize seeds. The Max Planck-Genome-centre Cologne (http:/ /mpgc.mpipz.mpg.de/home/) is acknowledged for performing Illumina sequencing. Moreover, we thank Kirsten Unger and Ingeborg Wieland for soil analyses and Judith Harz for excellent technical assistance.

References

Abarenkov, K., Henrik Nilsson, R., Larsson, K.-H., Alexander, I. J., Eberhardt, U., Erland, S., et al. (2010). The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytol.* 186, 281–285. doi:10.1111/j.1469-8137.2009.03160.x.

Ahn, J. H., Song, J., Kim, B. Y., Kim, M. S., Joa, J. H., and Weon, H. Y. (2012). Characterization of the bacterial and archaeal communities in rice field soils subjected to long-term fertilization practices. *J. Microbiol.* 50, 754–765. doi:10.1007/s12275-012-2409-6.

Azziz, G., Trasante, T., Monza, J., and Irisarri, P. (2016). The effect of soil type, rice cultivar and water management on ammonia-oxidizing archaea and bacteria populations. *Appl. Soil Ecol.* 100, 8–17. doi:https://doi.org/10.1016/j.apsoil.2015.11.009.

Badri, D. V., and Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell Environ.* 32, 666–681. doi:10.1111/j.1365-3040.2009.01926.x.

Bai, R., Wang, J. T., Deng, Y., He, J. Z., Feng, K., and Zhang, L. M. (2017). Microbial community and functional structure significantly varied among distinct types of paddy soils but responded differently along gradients of soil depth layers. *Front. Microbiol.* 8, 1–16. doi:10.3389/fmicb.2017.00945.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266. doi:10.1146/annurev.arplant.57.032905.105159.

Bastian, F., Bouziri, L., Nicolardot, B., and Ranjard, L. (2009). Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biol. Biochem.* 41, 262–275. doi:10.1016/j.soilbio.2008.10.024.

Bates, S. T., Berg-lyons, D., Caporaso, J. G., Walters, W. A., Knight, R., and Fierer, N. (2011). Examining the global distribution of dominant archaeal populations in soil. *ISME J.* 5, 908–917. doi:10.1038/ismej.2010.171.

Benitez, M.-S., Osborne, S. L., and Lehman, R. M. (2017). Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. *Sci. Rep.* 7, 15709. doi:10.1038/s41598-017-15955-9.

Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 68, 1–13. doi:10.1111/j.1574-6941.2009.00654.x.

Bernard, L., Mougel, C., Maron, P.-A., Nowak, V., Lévêque, J., Henault, C., et al. (2007). Dynamics and identification of soil microbial populations actively assimilating carbon from ^{13}C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. *Environ. Microbiol.* 9, 752–764. doi:10.1111/j.1462-2920.2006.01197.x.

Bilal, S., Khan, A. L., Shahzad, R., Asaf, S., Kang, S.-M., and Lee, I.-J. (2017). Endophytic Paecilomyces formosus LHL10 Augments Glycine max L. Adaptation to Ni-Contamination through Affecting Endogenous Phytohormones and Oxidative Stress. *Front. Plant Sci.* 8, 1–17. doi:10.3389/fpls.2017.00870.

Brefort, T., Doehlemann, G., Mendoza-Mendoza, A., Reissmann, S., Djamei, A., and Kahmann, R. (2009). *Ustilago maydis* as a Pathogen. *Annu. Rev. Phytopathol.* 47, 423–445. doi:10.1146/annurev-phyto-080508-081923.

Breidenbach, B., Blaser, M. B., Klose, M., and Conrad, R. (2015). Crop rotation of flooded rice with upland maize impacts the resident and active methanogenic microbial community. *Environ. Microbiol.* 18, 2868–2885. doi:10.1111/1462-2920.13041.

Breidenbach, B., and Conrad, R. (2015). Seasonal dynamics of bacterial and archaeal methanogenic communities in flooded rice fields and effect of drainage. *Front. Microbiol.* 5, 1–16. doi:10.3389/fmicb.2014.00752.

Brizzio, S., Turchetti, B., de García, V., Libkind, D., Buzzini, P., and van Broock, M. (2007). Extracellular enzymatic activities of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). *Can. J. Microbiol.* 53, 519–525. doi:10.1139/W07-010.

Brockett, B. F. T., Prescott, C. E., and Grayston, S. J. (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biol. Biochem.* 44, 9–20. doi:10.1016/j.soilbio.2011.09.003.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* 64, 807–838. doi:10.1146/annurev-arplant-050312-120106.

Cabangon, R. J., and Tuong, T. P. (2000). Management of cracked soils for water saving during land preparation for rice cultivation. *Soil Tillage Res.* 56, 105–116. doi:10.1016/S0167-1987(00)00125-2.

Castellanos, T., Dohrmann, A. B., Imfeld, G., Baumgarte, S., and Tebbe, C. C. (2009). Search of environmental descriptors to explain the variability of the bacterial diversity from maize rhizospheres across a regional scale. *Eur. J. Soil Biol.* 45, 383–393. doi:10.1016/j.ejsobi.2009.07.006.

Chen, H. G., Cao, Q. G., Xiong, G. L., Li, W., Zhang, A. X., Yu, H. S., et al. (2010). Composition of wheat rhizosphere antagonistic bacteria and wheat sharp eyespot as affected by rice straw mulching. *Pedosphere* 20, 505–514. doi:10.1016/S1002-0160(10)60040-9.

Colombo, R. P., Ibarra, J. G., Bidondo, L. F., Silvani, V. A., Bompadre, M. J., Pergola, M., et al. (2017). Arbuscular mycorrhizal fungal association in genetically modified drought-tolerant corn. *J. Environ. Qual.* 46, 227–231. doi:10.2134/jeq2016.04.0125.

Conrad, R. (2009). The global methane cycle: Recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* 1, 285–292. doi:10.1111/j.1758-2229.2009.00038.x.

Conrad, R., Klose, M., Lu, Y., and Chidthaisong, A. (2012). Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. *Front. Microbiol.* 3, 1–12. doi:10.3389/fmicb.2012.00004.

Crowther, T. W., Boddy, L., and Hefin Jones, T. (2012). Functional and ecological consequences of saprotrophic fungus-grazer interactions. *ISME J.* 6, 1992–2001. doi:10.1038/ismej.2012.53.

Da Rocha, U. N., van Overbeek, L., and van Elasa, J. D. (2013). Exploration of hitherto-uncultured bacteria from the rhizosphere. *Mol. Microb. Ecol. Rhizosph.* 1, 169–177. doi:10.1002/9781118297674.ch16.

Dick, R. P. (1992). A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agric. Ecosyst. Environ.* 40, 25–36. doi:10.1016/0167-8809(92)90081-L.

Dini-Andreote, F., Pylro, V. S., Baldrian, P., van Elsas, J. D., and Salles, J. F. (2016). Ecological succession reveals potential signatures of marine–terrestrial transition in salt marsh fungal communities. *ISME J.* 10, 1984–1997. doi:10.1038/ismej.2015.254.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996–998. doi:10.1038/nmeth.2604.

Edgar, R. C. (2016a). SINTAX : a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *Indep. Investig.* doi:10.1101/074161.

Edgar, R. C. (2016b). UCHIME2 : improved chimera prediction for amplicon sequencing. *Indep. Investig.* doi:10.1101/074252.

Edgar, R. C., and Flyvbjerg, H. (2015). Sequence analysis error filtering , pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 31, 3476–3482. doi:10.1093/bioinformatics/btv401.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., et al. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci.* 112, E911--E920. doi:10.1073/pnas.1414592112.

Fan, F., Yin, C., Tang, Y., Li, Z., Song, A., Wakelin, S. A., et al. (2014). Probing potential microbial coupling of carbon and nitrogen cycling during decomposition of maize residue by ^{13}C-DNA-SIP. *Soil Biol. Biochem.* 70, 12–21. doi:10.1016/j.soilbio.2013.12.002.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., et al. (2007). Changes in atmospheric constituents and in radiative forcing (IPCC 2007). *Clim. Chang. 2007 Phys. Sci. Basis. Contrib. Work. Gr. I to Fourth Assess. Rep. Intergov. Panel Clim. Chang.* 30, 129–234. doi:10.1103/PhysRevB.77.220407.

Gardes, G., and Bruns, T. (1993). ITS primers with enhanced specificity for *Basidiomycetes* - application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2, 113–118.

Geisseler, D., and Scow, K. M. (2014). Long-term effects of mineral fertilisers on soil microorganisms - A review. *Soil Biol. Biochem.* 75, 54–63. doi:10.1016/j.soilbio.2014.03.023.

Granzow, S., Kaiser, K., Wemheuer, B., Pfeiffer, B., Daniel, R., Vidal, S., et al. (2017). The Effects of Cropping Regimes on Fungal and Bacterial Communities of Wheat and Faba Bean in a Greenhouse Pot Experiment Differ between Plant Species and Compartment. *Front. Microbiol.* 8, 1–22. doi:10.3389/fmicb.2017.00902.

He, Y., Siemens, J., Amelung, W., Goldbach, H., Wassmann, R., Alberto, M. C. R., et al. (2015). Carbon release from rice roots under paddy rice and maize-paddy rice cropping. *Agric. Ecosyst. Environ.* 210, 15–24. doi:10.1016/j.agee.2015.04.029.

Hernández, M., Dumont, M. G., Yuan, Q., and Conrad, R. (2015). Different bacterial populations associated with the roots and rhizosphere of rice incorporate plant-derived carbon. *Appl. Environ. Microbiol.* 81, 2244–2253. doi:10.1128/AEM.03209-14.

Huo, C., Luo, Y., and Cheng, W. (2017). Rhizosphere priming effect: A meta-analysis. *Soil Biol. Biochem.* 111, 78–84. doi:10.1016/j.soilbio.2017.04.003.

Hussain, S., Siddique, T., Saleem, M., Arshad, M., and Khalid, A. (2009). "Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions," in Advances in Agronomy. (Academic Press), 159–200. doi:https://doi.org/10.1016/S0065-2113(09)01005-0.

Hyde, K. D., Jones, E. B. G., Liu, J.-K., Ariyawansa, H., Boehm, E., Boonmee, S., et al. (2013). Families of *Dothideomycetes*. *Fungal Divers.* 63, 1–313. doi:10.1007/s13225-013-0263-4.

Itoh, H., Ishii, S., Shiratori, Y., Oshima, K., Otsuka, S., Hattori, M., et al. (2013). Seasonal Transition of Active Bacterial and Archaeal Communities in Relation to Water Management in Paddy Soils. *Microbes Environ.* 28, 370–380. doi:10.1264/jsme2.ME13030.

Ke, X., Lu, W., and Conrad, R. (2015). High oxygen concentration increases the abundance and activity of bacterial rather than archaeal nitrifiers in rice field soil. *Microb. Ecol.* 70, 961–970. doi:10.1007/s00248-015-0633-4.

Kikuchi, H., Watanabe, T., Jia, Z., Kimura, M., and Asakawa, S. (2007). Molecular analyses reveal stability of bacterial communities in bulk soil of a Japanese paddy field: Estimation by denaturing gradient gel electrophoresis of 16S rRNA genes amplified from DNA accompanied with RNA. *Soil Sci. Plant Nutr.* 53, 448–458. doi:10.1111/j.1747-0765.2007.00177.x.

Kjøller, A. H., and Struwe, S. (2002). Fungal communities, succession, enzymes, and decomposition. *Enzym. Environ. Act. Ecol. Appl.* 1, 267–284.

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., et al. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *ISME J.* 6, 1378–1390. doi:10.1038/ismej.2011.192.

Ladygina, N., and Hedlund, K. (2010). Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biol. Biochem.* 42, 162–168. doi:10.1016/j.soilbio.2009.10.009.

Lee, C. G., Watanabe, T., Sato, Y., Murase, J., Asakawa, S., and Kimura, M. (2011). Bacterial populations assimilating carbon from ^{13}C-labeled plant residue in soil: Analysis by a DNA-SIP approach. *Soil Biol. Biochem.* 43, 814–822. doi:10.1016/j.soilbio.2010.12.016.

Letcher, P. M., Velez, C. G., Barrantes, M. E., Powell, M. J., Churchill, P. F., and Wakefield, W. S. (2008). Ultrastructural and molecular analyses of Rhizophydiales (Chytridiomycota) isolates from North America and Argentina. *Mycol. Res.* 112, 759–782. doi:10.1016/j.mycres.2008.01.025.

Li, X., Rui, J., Mao, Y., Yannarell, A., and Mackie, R. (2014). Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biol. Biochem.* 68, 392–401. doi:10.1016/j.soilbio.2013.10.017.

Liu, S., Huang, D., Chen, A., Wei, W., Brookes, P. C., Li, Y., et al. (2014). Differential responses of crop yields and soil organic carbon stock to fertilization and rice straw incorporation in three cropping systems in the subtropics. *Agric. Ecosyst. Environ.* 184, 51–58. doi:10.1016/j.agee.2013.11.019.

Lopes, A. R., Manaia, C. M., and Nunes, O. C. (2014). Bacterial community variations in an alfalfa-rice rotation system revealed by 16S rRNA gene 454-pyrosequencing. *FEMS Microbiol. Ecol.* 87, 650–663. doi:10.1111/1574-6941.12253.

Lu, Y., Rosencrantz, D., Liesack, W., and Conrad, R. (2006). Structure and activity of bacterial community inhabiting rice roots and the rhizosphere. *Environ. Microbiol.* 8, 1351–1360. doi:10.1111/j.1462-2920.2006.01028.x.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., et al. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488, 86–90. doi:10.1038/nature11237.

Maidak, B. L., Cole, J. R., Lilburn, T. G., Jr, C. T. P., Saxman, P. R., Stredwick, J. M., et al. (2000). The RDP (Ribosomal Database Project) continues. *Nucleic Acids Res.* 28, 173–174.

Martin, M. (2011). Cutadapt removes adapter sequences from high-thoughput sequencing reads. *EMBnet.journal* 17.1, 10–12. doi:dx.doi.org/10.14806/ej.17.1.200.

McMurdie, P. J., and Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* 8, 1–11. doi:10.1371/journal.pone.0061217.

Murase, J., Shibata, M., Lee, C. G., Watanabe, T., Asakawa, S., and Kimura, M. (2012). Incorporation of plant residue-derived carbon into the microeukaryotic community in a rice field soil revealed by DNA stable-isotope probing. *FEMS Microbiol. Ecol.* 79, 371–379. doi:10.1111/j.1574-6941.2011.01224.x.

Na Bhadalung, N., Suwanarit, A., Dell, B., Nopamornbodi, O., Thamchaipenet, A., and Rungchuang, J. (2005). Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. *Plant Soil* 270, 371–382. doi:10.1007/s11104-004-1829-4.

Nakamura, A., Tun, C. C., Asakawa, S., and Kimura, M. (2003). Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipid fatty acid analysis. *Biol. Fertil. Soils* 38, 288–295. doi:10.1007/s00374-003-0658-6.

Neufeld, J. D., and Mohn, W. W. (2005). Unexpectedly high bacterial diversity in arctic tundra relative to boreal forest soils , revealed by serial analysis of ribosomal sequence tags. *Appl. Environ. Microbiol.* 71, 5710–5718. doi:10.1128/AEM.71.10.5710.

Noll, M., Matthies, D., Frenzel, P., Derakshani, M., and Liesack, W. (2005). Succession of bacterial community structure and diversity in a paddy soil oxygen gradient. *Environ. Microbiol.* 7, 382–395. doi:10.1111/j.1462-2920.2004.00700.x.

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al. (2016). Vegan: Community ecology package. *R Packag.* 2.3-5.

Papizadeh, M., Soudi, M. R., Amini, L., Wijayawardene, N. N., and Hyde, K. D. (2017). *Pyrenochaetopsis tabarestanensis* (*Cucurbitariaceae*, *Pleosporales*), a new species isolated from rice farms in north Iran. *PHYTOTAXA* 297, 15–28. doi:10.11646/phytotaxa.297.1.2.

Parks, D. H., and Beiko, R. G. (2010). Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* 26, 715–721. doi:10.1093/bioinformatics/btq041.

Pascault, N., Ranjard, Kaisermann, A., Bachar, D., Christen, R., Terrat, S., et al. (2013). Stimulation of Different Functional Groups of Bacteria by Various Plant Residues as a Driver of Soil Priming Effect. *Ecosystems* 16, 810–822. doi:10.1007/s10021-013-9650-7.

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E., et al. (2016). Small but active - pool size does not matter for carbon incorporation in below-ground food webs. *Funct. Ecol.* 30, 479–489. doi:10.1111/1365-2435.12512.

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., et al. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci.* 110, 6548–6553. doi:10.1073/pnas.1302837110.

Phukhamsakda, C., Ariyawansa, H. A., Phillips, A. J. L., Wanasinghe, D. N., Bhat, D. J., McKenzie, E. H. C., et al. (2016). Additions to Sporormiaceae: Introducing Two Novel Genera, Sparticola and Forliomyces, from Spartium. *Cryptogam. Mycol.* v. 37, 75--97pp. 23--2016 v.37 no.1. doi:10.7872/crym/v37.iss1.2016.75.

Qiang, X., Weiss, M., Kogel, K. H., and Schäfer, P. (2012). *Piriformospora indica*-a mutualistic *basidiomycete* with an exceptionally large plant host range. *Mol. Plant Pathol.* 13, 508–518. doi:10.1111/j.1364-3703.2011.00764.x.

Qiao, Q., Wang, F., Zhang, J., Chen, Y., Zhang, C., Liu, G., et al. (2017). The variation in the rhizosphere microbiome of cotton with soil type, genotype and developmental stage. *Sci. Rep.* 7, 1–10. doi:10.1038/s41598-017-04213-7.

Reblova, M., and Winka, K. (2000). Phylogeny of Chaetosphaeria and Its Anamorphs Based on Morphological and Molecular Data. *Mycologia* 92, 939–954. doi:10.2307/3761589.

Rui, J., Peng, J., and Lu, Y. (2009). Succession of bacterial populations during plant residue decomposition in rice field soil. *Appl. Environ. Microbiol.* 75, 4879–4886. doi:10.1128/AEM.00702-09.

Semenov, A. V., Pereira e Silva, M. C., Szturc-Koestsier, A. E., Schmitt, H., Falcão Salles, J., and Van Elsas, J. D. (2012). Impact of incorporated fresh 13C potato tissues on the bacterial and fungal community composition of soil. *Soil Biol. Biochem.* 49, 88–95. doi:10.1016/j.soilbio.2012.02.016.

Shrestha, M., Shrestha, P. M., and Conrad, R. (2011). Bacterial and archaeal communities involved in the in situ degradation of ^{13}C-labelled straw in the rice rhizosphere. *Environ. Microbiol. Rep.* 3, 587–596. doi:10.1111/j.1758-2229.2011.00267.x.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Roskot, N., et al. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis : plant-dependent enrichment and seasonal shifts revealed bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* 67, 4742–4751. doi:10.1128/AEM.67.10.4742.

Tardy, V., Chabbi, A., Charrier, X., De Berranger, C., Reignier, T., Dequiedt, S., et al. (2015). Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. *PLoS One* 10, 1–17. doi:10.1371/journal.pone.0130672.

Thiele-Bruhn, S., Bloem, J., de Vries, F. T., Kalbitz, K., and Wagg, C. (2012). Linking soil biodiversity and agricultural soil management. *Curr. Opin. Environ. Sustain.* 4, 523–528. doi:https://doi.org/10.1016/j.cosust.2012.06.004.

Tuong, T. P., Bouman, B. a. M., and Mortimer, M. (2005). More rice, less water-integrated approaches for increasing water productivity in irrigated rice-based systems in asia. *Plant Prod. Sci.* 8, 231–241. doi:10.1626/pps.8.231.

Uroz, S., Oger, P., Tisserand, E., CéBron, A., Turpault, M. P., Bueé, M., et al. (2016). Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. *Sci. Rep.* 6, 1–11. doi:10.1038/srep27756.

Van Nguyen, N., and Ferrero, A. (2006). Meeting the challenges of global rice production. *Paddy Water Environ.* 4, 1–9. doi:10.1007/s10333-005-0031-5.

Vélez, C. G., Letcher, P. M., Schultz, S., Mataloni, G., Lefèvre, E., and Powell, M. J. (2013). Three new genera in *Chytridiales* from aquatic habitats in Argentina. *Mycologia* 105, 1251–1265. doi:10.3852/12-353.

Wang, B., Zhao, J., Guo, Z., Ma, J., Xu, H., and Jia, Z. (2015). Differential contributions of ammonia oxidizers and nitrite oxidizers to nitrification in four paddy soils. *ISME J.* 9, 1062–1075. doi:10.1038/ismej.2014.194.

Wang, J., Ni, L., Song, Y., Rhodes, G., Li, J., Huang, Q., et al. (2017). Dynamic response of ammonia-oxidizers to four fertilization regimes across a wheat-rice rotation system. *Front. Microbiol.* 8, 1–11. doi:10.3389/fmicb.2017.00630.

Wang, J., Wang, W., and Gu, J.-D. (2014). Community structure and abundance of ammonia-oxidizing archaea and bacteria after conversion from soybean to rice paddy in albic soils of Northeast China. *Appl. Microbiol. Biotechnol.* 98, 2765–2778. doi:10.1007/s00253-013-5213-2.

Wang, J., Xue, C., Song, Y., Wang, L., Huang, Q., and Shen, Q. (2016). Wheat and rice growth stages and fertilization regimes alter soil bacterial community structure, but not diversity. *Front. Microbiol.* 7, 1–13. doi:10.3389/fmicb.2016.01207.

Wang, Z., Binder, M., Schoch, C. L., Johnston, P. R., Spatafora, J. W., and Hibbett, D. S. (2006). Evolution of helotialean fungi (*Leotiomycetes, Pezizomycotina*): A nuclear rDNA phylogeny. *Mol. Phylogenet. Evol.* 41, 295–312. doi:10.1016/j.ympev.2006.05.031.

Weller, S., Kraus, D., Ayag, K. R. P., Wassmann, R., Alberto, M. C. R., Butterbach-Bahl, K., et al. (2015). Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. *Nutr. Cycl. Agroecosystems* 101, 37–53. doi:10.1007/s10705-014-9658-1.

White, T. J., Bruns, S., Lee, S., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. A Guid. to Methods Appl.*, 315–322. doi:citeulike-article-id:671166.

Zhang, L., Ma, H., Zhang, H., Xun, L., Chen, G., and Wang, L. (2015). Thermomyces lanuginosus is the dominant fungus in maize straw composts. *Bioresour. Technol.* 197, 266–275. doi:https://doi.org/10.1016/j.biortech.2015.08.089.

Zhang, T., Wang, N. F., Zhang, Y. Q., Liu, H. Y., and Yu, L. Y. (2016). Diversity and Distribution of Aquatic Fungal Communities in the Ny-Ålesund Region, Svalbard (High Arctic): Aquatic Fungi in the Arctic. *Microb. Ecol.* 71, 543–554. doi:10.1007/s00248-015-0689-1.

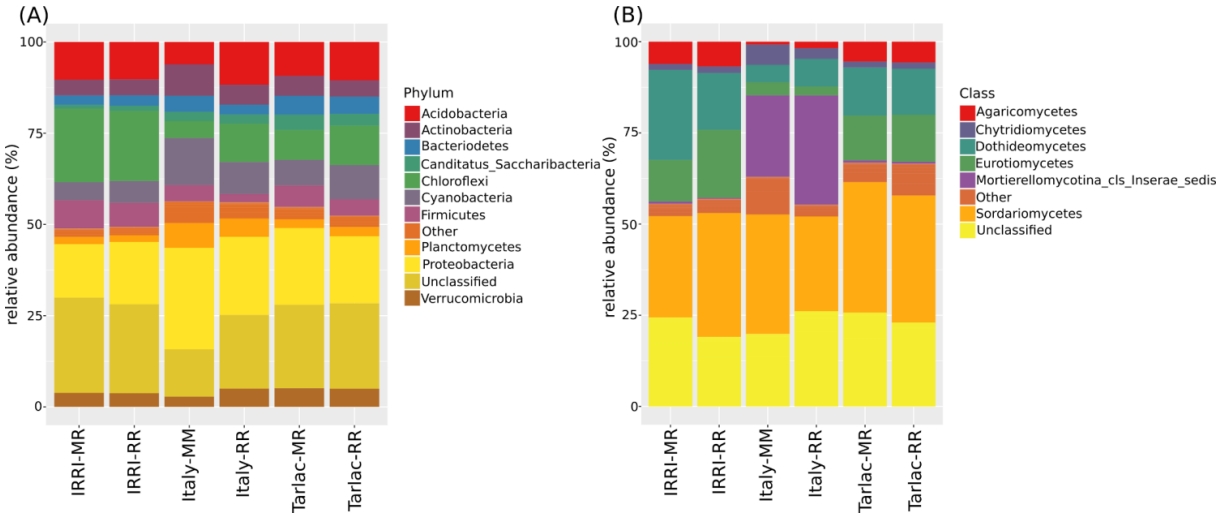
Zhao, J., Zhang, R., Xue, C., Xun, W., Sun, L., Xu, Y., et al. (2014). Pyrosequencing reveals contrasting soil bacterial diversity and community structure of two main winter wheat cropping systems in china. *Microb. Ecol.* 67, 443–453. doi:10.1007/s00248-013-0322-0.

Zhao, M., Sun, B., Wu, L., Gao, Q., Wang, F., Wen, C., et al. (2016). Zonal soil type determines soil microbial responses to maize cropping and fertilization. *mSystems* 1, e00075--16. doi:10.1128/mSystems.00075-16.

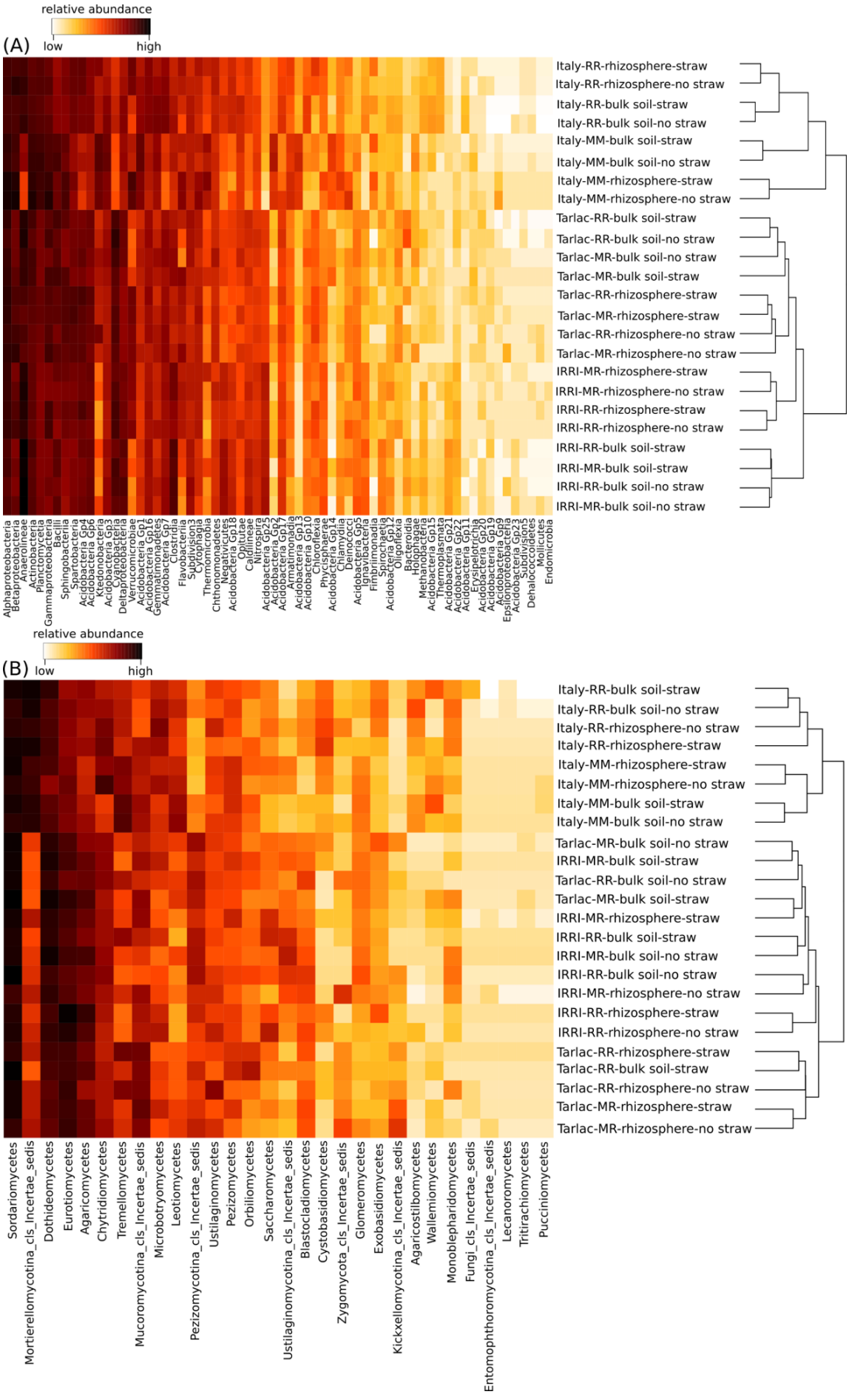
Zhao, Z., Liu, H., Wang, C., and Xu, J.-R. (2013). Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. *BMC Genomics* 14, 274. doi:10.1186/1471-2164-14-274.

Supplementary figures and tables

Supplementary figures

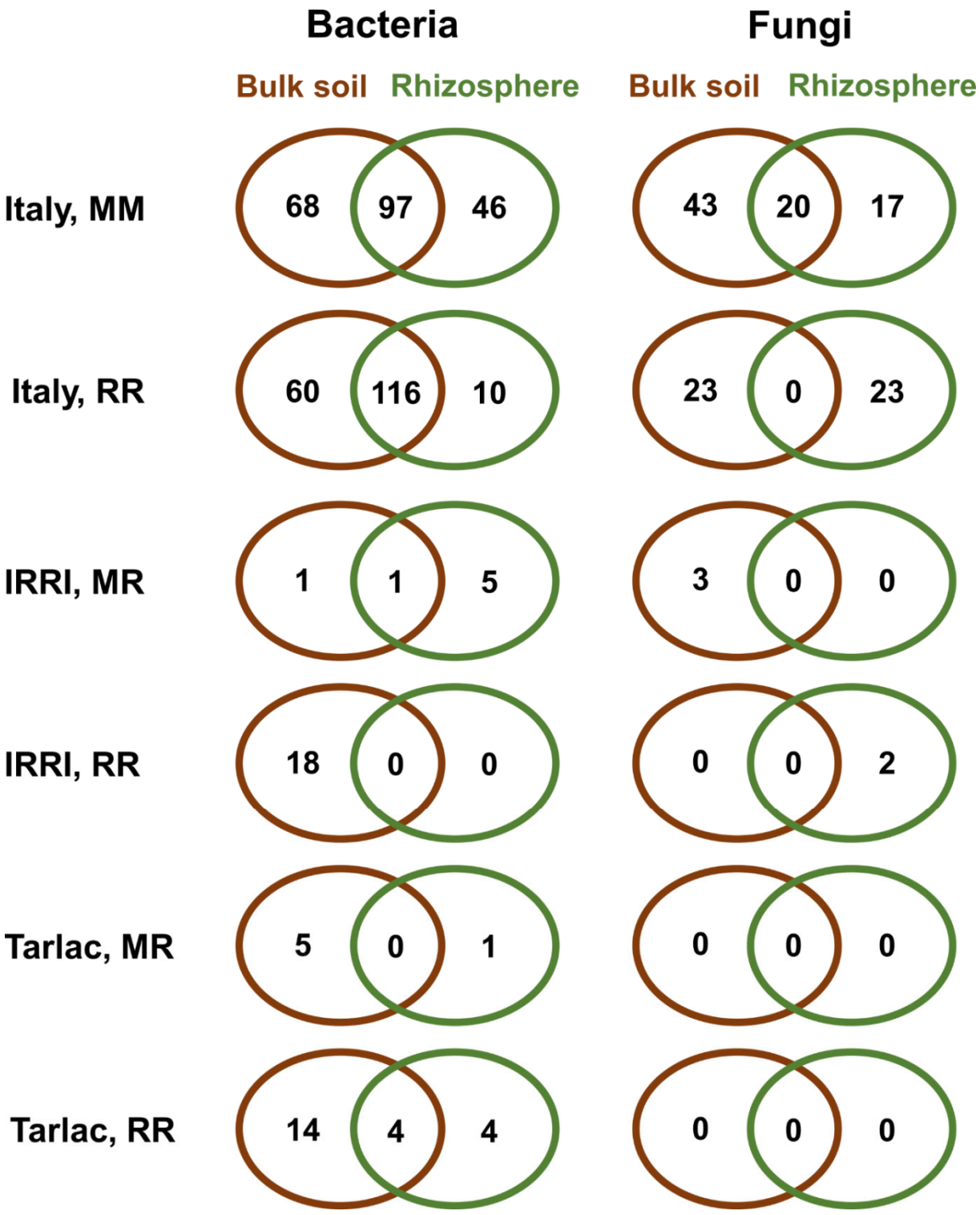


Supplementary Figure 1: Stacked bar diagrams showing the relative abundance of bacterial phyla (A) and fungal classes (B) in samples from fields undergoing different crop rotation regimes. Phyla and classes with < 2 % relative abundance are grouped and displayed as “Other”.

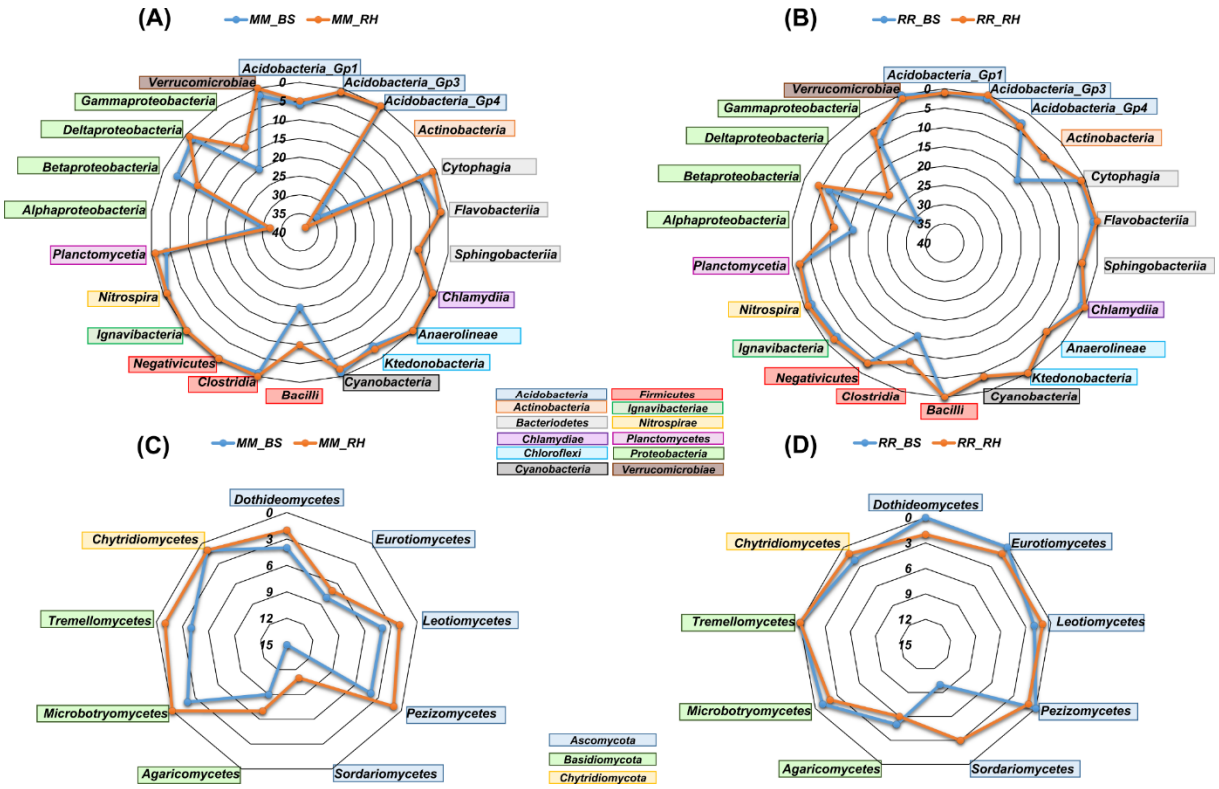

relative abundance
low
high
(A)
Italy-RR-rhizosphere-straw
Italy-RR-rhizosphere-no straw
Italy-RR-bulk soil-straw
Italy-RR-bulk soil-no straw
Italy-MM-bulk soil-straw
Italy-MM-bulk soil-no straw
Italy-MM-rhizosphere-straw
Italy-MM-rhizosphere-no straw
Tarlac-RR-bulk soil-straw
Tarlac-RR-bulk soil-no straw
Tarlac-MR-bulk soil-no straw
Tarlac-MR-bulk soil-straw
Tarlac-RR-rhizosphere-straw
Tarlac-MR-rhizosphere-straw
Tarlac-RR-rhizosphere-no straw
Tarlac-MR-rhizosphere-no straw
IRRI-MR-rhizosphere-straw
IRRI-MR-rhizosphere-no straw
IRRI-RR-rhizosphere-straw
IRRI-RR-rhizosphere-no straw
IRRI-RR-bulk soil-straw
IRRI-MR-bulk soil-straw
IRRI-RR-bulk soil-no straw
IRRI-MR-bulk soil-no straw
Alphaproteobacteria
Betaproteobacteria
Anaerolineae
Actinobacteria
Planctomycetia
Gammaproteobacteria
Bacilli
Sphingobacteriia
Spartobacteria
Acidobacteria Gp4
Acidobacteria Gp6
Ktedonobacteria
Acidobacteria Gp3
Deltaproteobacteria
Verrucomicrobiae
Acidobacteria Gp16
Acidobacteria Gp1
Gemmatimonadetes
Acidobacteria Gp7
Clostridia
Flavobacteriia
Subdivision3
Cytophagia
Thermomicrobia
Chthonomonadetes
Negativicutes
Acidobacteria Gp18
Opitutae
Caldilineae
Nitrospirae
Acidobacteria Gp25
Acidobacteria Gp2
Acidobacteria Gp17
Alphaproteobacteria
Acidobacteria Gp13
Acidobacteria Gp10
Chloroflexia
Phycisphaerae
Acidobacteria Gp14
Chlamydiae
Deinococci
Acidobacteria Gp5
Ignavibacteria
Fimbriimonadia
Spirochaetia
Acidobacteria Gp12
Oligoflexia
Bacteroidia
Holophagae
Methanobacteria
Acidobacteria Gp15
Thermoplasmata
Acidobacteria Gp21
Acidobacteria Gp22
Actinobacteria
Erysipelotrichia
Acidobacteria Gp20
Acidobacteria Gp19
Acidobacteria Gp9
Epsilonproteobacteria
Acidobacteria Gp23
Subdivision5
Dehalococcoidetes
Mollicutes
Endomicrobia
relative abundance
low
high
(B)
Italy-RR-bulk soil-straw
Italy-RR-bulk soil-no straw
Italy-RR-rhizosphere-no straw
Italy-RR-rhizosphere-straw
Italy-MM-rhizosphere-straw
Italy-MM-rhizosphere-no straw
Italy-MM-bulk soil-straw
Italy-MM-bulk soil-no straw
Tarlac-MR-bulk soil-no straw
IRRI-MR-bulk soil-straw
Tarlac-RR-bulk soil-no straw
Tarlac-MR-bulk soil-straw
IRRI-MR-rhizosphere-straw
IRRI-RR-bulk soil-straw
IRRI-MR-bulk soil-no straw
IRRI-RR-bulk soil-no straw
IRRI-MR-rhizosphere-no straw
IRRI-RR-rhizosphere-straw
IRRI-RR-rhizosphere-no straw
Tarlac-RR-rhizosphere-straw
Tarlac-RR-bulk soil-straw
Tarlac-RR-rhizosphere-no straw
Tarlac-MR-rhizosphere-straw
Tarlac-MR-rhizosphere-no straw
Sordariomycetes
Mortierellomycotina_cls_Incertae_sedis
Dothideomycetes
Eurotiomycetes
Agaricomycetes
Chytridiomycetes
Tremellomycetes
Mucoromycotina_cls_Incertae_sedis
Microbotryomycetes
Leotiomycetes
Pezizomycotina_cls_Incertae_sedis
Ustilaginomycetes
Pezizomycetes
Orbiliomycetes
Saccharomycetes
Ustilaginomycotina_cls_Incertae_sedis
Blastocladiomycetes
Cystobasidiomycetes
Zygomycota_cls_Incertae_sedis
Glomeromycetes
Exobasidiomycetes
Kickxellomycotina_cls_Incertae_sedis
Agaricostilbomycetes
Wallemiomycetes
Monoblepharidomycetes
Fungi_cls_Incertae_sedis
Entomophthoromycotina_cls_Incertae_sedis
Lecanoromycetes
Tritirachiomycetes
Pucciniomycetes

Supplementary figure 2: Sample clustering of bacterial (A) and fungal (B) communities according to the UPGMA algorithm based on Bray-Curtis dissimilarities between groups of samples. The heat maps show log(x+1) transformed relative abundances of the bacterial and fungal classes, sorted by decreasing relative abundance. For the heat map, the OTUs were grouped at class level. Samples representing different time points were grouped and relative abundances were calculated based on summarized read numbers. Unclassified OTUs were excluded from the analysis. The heatmap was constructed in R using the package Heatmap3.

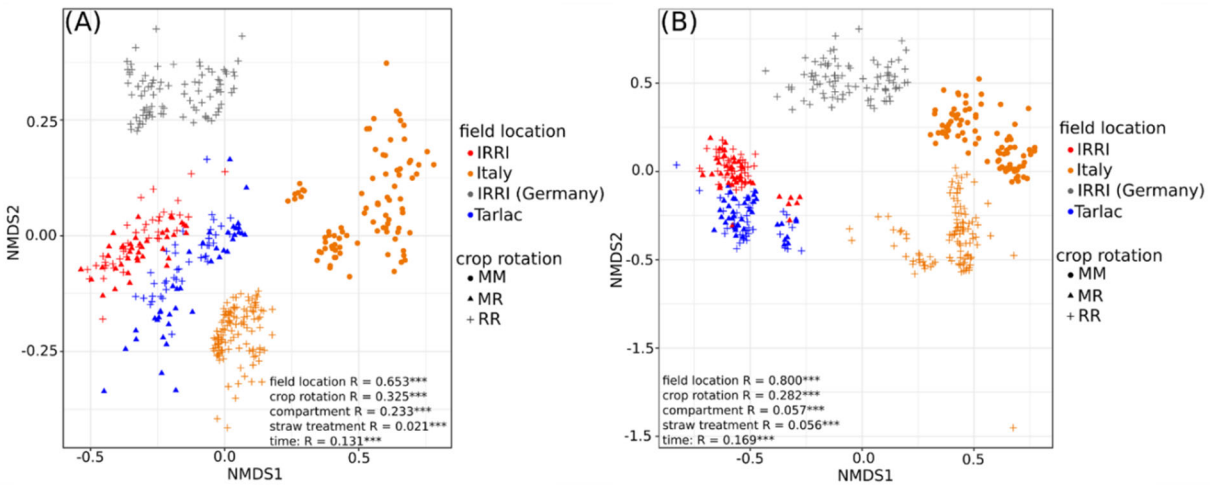
The clustering of groups of samples reveals that bacterial and fungal communities were well separated according to field location, and the most distinct samples were those from the Italian field sites. A clear separation according to field location was also observed for the bacterial communities in the soils from the two different Philippine sites, but this separation was weaker for the fungal communities. The impact of crop rotation was of particular strength in the Italian soils. The differences between compartments are evident, especially in the Italian soils.



Supplementary Figure 3: Venn diagrams displaying numbers of compartment specific and compartment-independently enriched bacterial and fungal genera in dependence on crop rotation. The impact of crop rotation was analyzed in MM versus RR (Italy) or MR versus RR soils (IRRI and Tarlac) using the STAMP algorithm (list of genera in supplementary tables 6 C, D).



Supplementary Figure 4: Radar charts showing the number of bacterial (A), (B) and fungal (C), (D) genera in the different classes that were identified as significantly enriched by crop rotation in the different compartments (BS = bulk soil, RH = rhizosphere) based on STAMP analysis. Plots are shown for Italian MM soil (A), (C) and RR soil (B), (C). Displayed are classes for which at least three different genera were identified as specifically enriched in one or the other soil.



Supplementary Figure 5: Ordination plots showing the influence of field location, crop rotation, compartment, straw treatment and time on bacterial (A) and fungal (B) community composition. NMDS plots based on Bray-Curtis similarities were calculated based on relative OTU abundance. Results of ANOSIM are shown with $P < 0.05$*, $P < 0.01$**, $P < 0.001$*** for all grouping factors.

In comparison to figure 1, these plots include results obtained from "IRRI (Germany)" samples, which were collected earlier at the IRRI site from RR soils, shipped to Germany and included in the microcosm experiment performed with soils from Italy. This was done to evaluate the potential impact of the experimental study site location and the maize cultivar. The plots reveal that IRRI (Germany) samples cluster distinctly but still closely to those from IRRI, especially in case of the bacterial community. This demonstrates that we cannot exclude that the conductance of the microcosm experiments at two different locations has contributed to some extent to the observed differences between the Italian and Philippine field sites. However, the still very distinct clustering of IRRI (Germany) samples from Italy samples demonstrates that other site-specific factors contributed more substantially to the site-specific differences. A major effect of the maize cultivar can be excluded, as the rhizosphere samples clustered in all cases very closely together with the corresponding bulk soil samples in these plots.

Supplementary tables

Supplementary Table 1: Soil parameters of homogenized soil samples, analysed before the start of the experiment. Measurements of N_{min}, C/N and clay fraction were performed in duplicates. Mean values ± standard error are shown.

	pH	N_{min} (mg/kg)	N (%)	C (%)	C:N	C_{org} (%)	Soil type	Clay fraction (%)	Water holding capacity (%)
Italy RR	4.9	24.97 ± 1.79	0.07	0.98	12.71 ± 0.24	0.98	loam	9.51 ±0.47	41.8
Italy MM	4.2	27.63 ± 0.46	0.06	0.74	11.11 ± 0.89	0.75	sandy loam	13.22 ±0.34	43.9
IRRI RR	5.7	6.81 ± 0.14	0.14	1.73	11.89 ± 0.51	1.74	silty clay	59.56 ±0.35	79.9
IRRI MR	5.7	4.71 ± 0.05	0.15	1.81	11.94 ± 0.86	1.82	silty clay	60.17 ± 0.04	72.7
Tarlac RR	5.8	3.89 ± 0.02	0.06	0.77	11.67 ± 0.22	0.77	loam	10.19 ± 0.08	60.3
Tarlac MR	5.2	4.40 ± 0.09	0.06	0.95	13.96 ± 0.01	0.96	silty loam	12.87 ± 0.09	53.9

Supplementary Table 2: Richness and diversity of fungal and bacterial communities. Numbers represent mean values ± standard deviation.

		Bacteria				Fungi			
Treatments		**Richness**	***P*-value**	**Chao1 diversity**	***P*-value**	**Richness**	***P*-value**	**Chao1 diversity**	***P*-value**
Field location	Italy	1221 ± 253	< 0.001	1974 ± 253	< 0.001	176 ± 49		316 ± 113	< 0.001
	IRRI	1347 ± 135		1984 ± 255		198 ± 45		314 ± 76	
	Tarlac	1523 ± 201		2386 ± 389		214 ± 53		373 ± 106	
Crop rotation	RR	1401 ± 192	< 0.001	2221 ± 368	< 0.001	195 ± 52	< 0.001	348 ± 112	< 0.001
	MM	1022 ±209		1641 ± 431		155 ± 28		260 ± 64	
	MR	1417 ± 172		2138 ± 345		217 ± 50		357 ± 100	
Compartment	bulk soil	1396 ± 212	< 0.001	2188 ± 397	< 0.001	199 ± 47	< 0.001	310 ± 104	< 0.001
	rhizosphere	1207 ± 260		1903 ± 460		178 ± 55		300 ± 104	
Straw treatment	no straw	1297 ± 245	0.015	2035 ± 436	0.018	207 ± 51	< 0.001	356 ± 109	< 0.001
	straw	1331 ± 241		2094 ± 434		173 ± 46		301 ± 97	
Time	0	1519 ± 156	< 0.001	2387 ± 328	< 0.001	208 ± 50	< 0.001	372 ± 130	< 0.01
	8	1212 ± 207		1960 ± 434		175 ± 54		322 ± 123	
	15	1307 ± 202		2026 ± 376		183 ± 42		306 ± 83	
	29	1163 ± 287		1883 ± 502		166 ± 38		290 ± 80	
	43	1347 ± 275		2057 ± 487		206 ± 59		348 ± 111	
	85	1129 ± 239		1786 ± 433		154 ± 24		250 ± 52	

Supplementary Table 3: Influence of crop rotation on microbial community composition according to R-values derived from an ANOSIM. P < 0.001***, P < 0.01**, P < 0.05*.

	Time	0	8	15	29	43	85
16S rRNA gene sequence data	Italy bulk soil	1***	1***	1***	1***	1***	1***
	Italy rhizosphere		1***	1***	0.936***	1***	1***
	IRRI bulk soil	0.456**		0.531***		0.536***	
	IRRI rhizosphere			0.826***		0.287**	
	Tarlac bulk soil	0.442**		0.486***		0.143*	
	Tarlac rhizosphere			0.462***		0.510***	
ITS1 sequence data	Italy bulk soil	0.509***	0.882***	0.793***	0.865***	0.843***	0.714***
	Italy rhizosphere		0.119*	0.514***	0.950***	0.428***	0.766***
	IRRI bulk soil	0.153*		0.164*		0.174*	
	IRRI rhizosphere			0.282**		0.310**	
	Tarlac bulk soil	0.189**		0.217**		0.214**	
	Tarlac rhizosphere			0.222**		0.436***	

The calculation of mean R-values from this table and comparison by ANOVA demonstrated that the response to crop rotation was significantly stronger in Italian soils than in Philippine soils ($P < 0.001$) and that fungal communities showed a stronger response than bacterial communities (paired t-test, $P < 0.005$).

Supplementary Table 4: Influence of straw mulching on microbial community composition according to R-values derived from an ANOSIM. P < 0.05*.

		Time	0	8	15	29	43	85
16S rRNA gene sequence data	Bulk soil	Italy RR	0.073	0.583*	0.438*	0.198*	0.219*	0.677*
		Italy MM	0.063	0.542*	0.406*	0.260*	0.260*	0.468*
		IRRI RR	0.260*		0.031		0.960*	
		IRRI MR	0.239*		-0.125		0.510*	
		Tarlac RR	0.146		0.125		-0.010	
		Tarlac MR	0.615*		0.072		0.135	
	Rhizosphere	Italy RR		-0.021	0.844*	0.448*	0.388*	0.760*
		Italy MM		0.406*	0.635*	0.698*	0.593*	0.135
		IRRI RR			0.292*		-0.063	
		IRRI MR			-0.073		0.844*	
		Tarlac RR			0.615*		0.427*	
		Tarlac MR			1*		0.885*	
ITS1 sequence data	Bulk soil	Italy RR	0.583*	0.729*	0.854*	0.365*	0.323*	0.385*
		Italy MM	0.083	0.500*	0.395*	0.177*	0.562*	0.281
		IRRI RR	0.970*		-0.031		0.948*	
		IRRI MR	0.281*		0.521*		0.447*	
		Tarlac RR	0.896*		0.354		0.656*	
		Tarlac MR	0.875*		0.348		-0.021	
	Rhizosphere	Italy RR		0.479*	0.062	-0.166	0.479*	1*
		Italy MM		0.656*	0.604*	1*	0.875*	1*
		IRRI RR			0.646*		0.156	
		IRRI MR			0.177*		1*	
		Tarlac RR			0.218		0.083	
		Tarlac MR			0.792*		1*	

The calculation of mean R-values from this table (including only significant and thus reliable R-values) and comparison by ANOVA demonstrated that fungal communities showed a stronger response than bacterial communities ($P < 0.05$) and that responses were stronger in the rhizosphere than in bulk soil ($P < 0.01$).

Supplementary Table 5: Influence of straw treatment and time point of sampling on bacterial and fungal community composition. R-values based on ANOSIM are presented with P < 0.001***, P < 0.01**, P < 0.05*.

	Bacteria		Fungi	
	Straw	**Time**	**Straw**	**Time**
Italy RR bulk soil	0.088**	0.472***	0.304***	0.145***
Italy RR rhizosphere	0.107**	0.684***	0.330***	0.326***
Italy MM bulk soil	0.109**	0.444***	0.181***	0.045
Italy MM rhizosphere	0.051	0.691***	0.645***	0.222***
IRRI RR bulk soil	0.055	0.569***	0.191**	0.352**
IRRI RR rhizosphere	0.001	0.696**	0.046	0.119**
IRRI MR bulk soil	-0.011	0.579***	0.223**	0.188**
IRRI MR rhizosphere	0.075	0.913***	0.410**	0.312**
Tarlac RR bulk soil	0.050	0.229***	0.320**	0.249**
Tarlac RR rhizosphere	0.175*	0.605***	0.103	0.479***
Tarlac MR bulk soil	0.085	0.310***	0.374***	0.139*
Tarlac MR rhizosphere	0.528**	0.806***	0.694***	0.463***

The Supplementary table 6 of this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb. 2018.01295/full#supplementary-material.

2.3. Temporal dynamic and compartment specific rice straw degradation in bulk soil and the rhizosphere of maize (manuscript 3)

Sarah A. Maarastawi[1], Katharina Frindte [1], Romy Geer[1], Eileen Kröber[2], Claudia Knief [1,*]

[1]Institute for Crop Science and Resource Conservation (INRES), Molecular Biology of the Rhizosphere, University of Bonn, Bonn, Germany

[2]Landscape Functioning, Microbial Biogeochemistry, Leibniz Centre for Agricultural Landscape Research (ZALF)

Author contributions:

SM and CK conceived the research. SM designed the research. SM and RG performed the experiments. SM analysed the data with help of EK and KF. SM wrote the paper. SM, KF and CK approved the paper.

Published in *Soil Biology and Biochemistry* (2018) doi.org/10.1016/j.soilbio.2018.09.028

Abstract

Crop rotation between paddy rice and maize is of increasing relevance because of ecological and economic reasons. However, introduction of upland crops in paddy fields often leads to carbon and nitrogen loss due to desiccation crack formation. Straw application can reduce crack formation and acts as fertilizer. The temporal dynamics of straw degradation under oxic conditions in paddy soils undergoing crop rotation have been scarcely studied. We identified the straw degrading microorganisms comparatively in the bulk soil and rhizosphere of maize by DNA-stable isotope probing with ^{13}C-labelled rice straw and amplicon sequencing of the 16S rRNA gene and ITS1 region. Moreover, the degradation process in bulk soil was investigated over time. Straw degradation was performed by aerobic microorganisms and showed a clear temporal succession. In the initial phase, fast growing bacteria became labelled, followed by the labelling of fungi, known to degrade more complex carbon compounds. In the rhizosphere, partly different microorganisms were identified as labelled than in bulk soil, indicating that the rhizosphere hosts specific taxa that benefit from straw. The label intensity in the rhizosphere was lower than in bulk soil, indicating that rhizosphere microorganisms use straw as additional carbon source with lower efficiency besides the rhizodeposits.

Introduction

Crop rotation regimes alternating between rice cultivation during the wet season and maize cultivation during the dry season represent an interesting alternative to rice monoculture systems in Asia. They allow to reduce water consumption and methane emissions and to meet the increased demand of maize for poultry fattening and biofuel production (Fuhrmann et al., 2018; Qiu et al., 2009; Tuong et al., 2005). However, crop rotation can lead to crack formation and hence to increasing emissions of the greenhouse gas nitrous oxide as well as a loss of water, carbon and nitrogen (He et al., 2015; Weller et al., 2015). Straw mulching is considered to reduce crack formation and thus to maintain soil moisture (Cabangon and Tuong, 2000).

The effect of rice straw application on microbial communities has been investigated in paddy soils under rice monoculture, as rice straw application is a traditional fertilization practice (Asari et al., 2007). Shrestha *et al.*, (2011) and Conrad *et al.* (2012) showed that the introduction and degradation of rice straw in paddy soil under flooded conditions leads to increased methane (CH_4) production and an alteration in bacterial and archaeal community composition. The degradation of rice straw under anoxic conditions results in the formation of acetate, formate and H_2 as intermediate products (Shrestha et al., 2011). Further degradation of these products leads to the formation of carbon dioxide (CO_2) and CH_4 as final products through various fermenting bacteria and methanogenic archaea. A significantly higher CH_4 production by methanogens was observed upon straw addition in bulk soil of paddy soil as well as in the rice rhizosphere in comparison to soil incubated without straw (Shrestha et al., 2011; Watanabe et al., 1998). Besides methanogens, several bacterial taxa like *Clostridium*, *Proteobacteria*, *Bacteroidetes*, *Chlorobi*, *Acidobacteria*, *Actinobacteria*, *Sphingobacteria*, *Cyanobacteria* and *Bacilli* were identified as part of the straw degrading community in bulk soil of flooded rice soils (Rui et al., 2009). Interestingly, Lee et al. (2012) observed growth of acetoclastic and hydrogenotrophic methanogens in rice field soil even under non-flooded conditions upon rice straw application. The microbial community involved in straw degradation in paddy soil undergoing crop rotation, which is exposed to non-flooded conditions for a longer period of time than paddy soil managed under monoculture, was not investigated yet. Thus, the question about the microorganisms involved in the straw degradation process arises and whether anaerobic microorganisms are still involved in this process in such soils.

Rice straw consists of rather easily degradable carbon compounds, e. g. polysaccharides such as cellulose and hemicellulose, which serve as valuable carbon source for different soil microorganisms, as well as of more complex compounds such as lignin, which is only degraded by specialized microorganisms (Watanabe et al., 1993). Pascault et al. (2013) and Bernard et

al. (2007) observed that the addition of straw leads to a rapid and durable stimulation of copiotrophic fresh organic matter (FOM) degrading populations. Copiotrophs induced the straw degradation process, because they preferentially consume labile organic carbon and show high growth rates when resources are abundant. In contrast, oligotrophs became involved in the straw decomposition process at later time points, because they exhibit slower growth rates and are able to degrade more complex straw components, which are not readily metabolised by the copiotrophs (Fierer et al., 2007; Meyer, 1994). Thus, microbial groups involved in FOM degradation undergo a temporal succession. To identify the microorganisms involved in straw degradation in non-flooded paddy soil, it is thus important to investigate the process at different time points.

The bulk soil and rhizosphere are characterized by different physicochemical conditions, and it is generally accepted that rhizosphere microorganisms benefit from root released carbon (Hinsinger et al., 2009; Philippot et al., 2013). Shrestha *et al.* (2011) were the first who analysed the straw degrading bacterial and archaeal community in the rhizosphere of rice and observed that mainly members of *Clostridium* cluster I, III and XIVa as well as methanogens like *Methanosarcina* were responsible for straw degradation in the rhizosphere. It can be expected that microorganisms involved in rice straw degradation in the rhizosphere are to some extent different to those in bulk soil, but studies evaluating this aspect are lacking.

The aim of this study was to investigate the identity and temporal succession of the straw degrading bacterial and fungal communities in a paddy soil undergoing rice-maize crop rotation during the period of maize cultivation. Moreover, we addressed the question whether the rhizosphere microbiota profits from straw derived carbon and whether a different community is involved in the process in comparison to the bulk soil. We hypothesized that i) straw degradation under oxic conditions in a paddy soil undergoing crop rotation is mainly conducted by aerobic microbes and to less extent by anaerobic microorganisms ii) straw degradation undergoes a temporal succession and can be classified into different stages, according to the stimulation of different taxa at different stages of straw degradation iii) the active straw degrading community in the rhizosphere differs from that in the bulk soil. To verify these hypotheses, we performed a microcosm experiment, in which we added >97 atom-% labelled ^{13}C rice straw to soil from a paddy field undergoing rice-maize crop rotation since four years. The active straw degrading microbial community in bulk soil and rhizosphere samples was identified using a DNA stable isotope probing (SIP) approach in combination with amplicon sequencing of the bacterial and fungal marker genes, i. e. the 16S rRNA gene and ITS1 region, respectively.

Material and methods

Study site and experimental set up

The soil sampling site was located at the International Rice Research Institute (IRRI) in Los Banos, Philippines (14°11'N, 121°15'E). For the microcosm experiment, soil was taken from a field that was managed under crop rotation for four years, with maize (variety Pioneer 90T80) in the dry season and paddy rice (variety RC 222) in the wet season. The microcosm experiment was conducted in a greenhouse at IRRI during the dry season in December 2016. Before the start of the experiment, soil was air dried and well homogenized. The soil was re-moistened, mixed with unlabelled milled rice straw (6 kg straw * m^{-3}) and used to fill plastic pots up to 1.2 L. For the bulk soil labelling experiment, 20 g of dry weight soil was mixed with 0.13 g (corresponding to 6 kg straw * m^{-3}) ^{13}C-labelled straw and put into a nylon bag (11 µm mesh size). Bags were buried in the middle of the pots, which had been filled with the homogenized soil and unlabelled straw. Nearly fully labelled ^{13}C-straw (*Oryza sativa* straw, > 97 atom % ^{13}C, C : N ratio = 40) was obtained from Isolife bv (Wageningen, The Netherlands). For the rhizosphere samples, pots were filled with 1.2 l soil, which was mixed with either ^{13}C-labelled straw or unlabelled straw, and maize (variety Pioneer 90T80) was sown. Rhizosphere samples were collected 21 days after seeding. Bulk soil sampling started at time point 0, followed by sampling after 3, 7, 14, 21, 28 and 35 days. The experiment was conducted in triplicates for all treatments (maize with ^{13}C-labelled and unlabelled straw, bulk soil with ^{13}C-labelled and unlabelled straw) and every time point. The maize plants and bulk soil pots were watered every day and received basal fertilization of 50 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} at seeding. Nitrogen was used for fertilization in three split applications with 30 kg urea ha^{-1} basal fertilization at seeding and 50 kg urea ha^{-1} at 29 days after seeding. Collection of the soil and rhizosphere samples and storage until further analyses was done as described before (Maarastawi et al., 2018).

Nucleic acid extraction

DNA extraction from soil samples was performed using a modified phenol chloroform protocol according to Töwe *et al.* (2011). To obtain sufficient DNA for DNA-SIP from the soil samples, which had a very high clay content, a pre-extraction with sodium metaphosphate (SMP) was performed according to (Pietramellara et al., 2001). Therefore, 0.6 g of moist soil was mixed with 700 µL of nuclease-free water, 100 µL of 0.1 M SMP and 0.5 g of zirconium silicon beads. Beat beating was performed for 2 min at 1800 rpm using a Fastprep96™ instrument (MP Biomedicals, Germany). After centrifugation for 1 min at 10.000 x g and

4 °C, the supernatant was transferred to a new tube, while the pellet was resuspended in 600 µL of nuclease-free water and 100 µL of 0.1 M SMP. Beat beating was repeated for 1 min, the sample centrifuged and the supernatant transferred to a fresh tube, resulting in two extracts per sample. For the further extraction according to Töwe *et al.* (2011), 550 µL of hexadecyltrimethylammonium bromide (CTAB) extraction buffer, 55 µL of 10 % sodium dodecyl sulfate, 55 µL of 10 % lauroyl sarcosine, 550 µL of phenol: chloroform: isoamyl alcohol (25:24:1) (pH 7.5) and 0.5 g of zirconium silicon beads were added to the supernatant. After another beat beating step for 1 min at 1800 rpm, which was introduced to increase DNA recovery, the samples were centrifuged for 10 min at 16.000 x g and 4°C. The upper, aqueous phase was transferred into a new tube and the chloroform extraction as well as the precipitation step was conducted according to Töwe *et al.* (2011). Pellets of the same sample were combined during the ethanol washing step. Therefore, one of the two pellets was resuspended in 700 µL ice cold 96 % ethanol and the suspension transferred to the second tube. To ensure complete transfer of the DNA, the procedure was repeated with 400 µL of ice cold 70 % ethanol. After centrifugation (10 min at maximum speed) and removal of the supernatant, the pellets were air-dried, the DNA resuspended in 30 µL of nuclease-free water and stored at -20 °C.

Isopycnic centrifugation, gradient fractionation and DNA precipitation

Gradient preparation and density centrifugation were performed according to Lueders *et al.*, 2004. DNA was spun in a CsCl gradient with an average density of 1.72 g mL^{-1}. The centrifugation medium consisted of 4.8 mL CsCl (1.85 g mL^{-1}), 1 mL gradient buffer (1 mM EDTA, 0.1 M Tris-HCL (pH 8.0) and 0.1 M KCl) and DNA extract (2 µg DNA). The samples were centrifuged in 5.1 mL polypropylene quick-seal tubes in a VTI 65.2 vertical rotor using the Optima XPN 80 centrifuge (all from Beckmann Coulter Inc., USA). Centrifugation was performed for 39 h at 177.000 x g and 20 °C. Soil samples from triplicate ^{12}C-treatments were pooled, extracted and fractionated as a composite sample, likewise as done by Kramer et al. (2016), whereas samples treated with ^{13}C-rice straw were kept as individual replicates per time point and compartment. Before ^{12}C-sample pooling, the homogeneity of the replicates was validated based on denaturing gradient gel electrophoresis (DGGE) (data not shown).

Gradient fractionation was performed according Neufeld *et al.*, (2007) with minor modifications. Gradients were fractionated using a peristaltic pump (ISM850, Ismatec, Switzerland) with a flow of approximately 480 µL min^{-1} to fractionate samples into 12 equal fractions of approximately 400 µL. Displacement of the gradient medium was done by injecting nuclease-free water coloured with loading dye (0.25% bromphenol blue, 5 mM NaOH, 4 M

urea). The density was measured refractometrically with an AR200 digital refractometer (Reichert, USA) using 50 µl of each fraction.

For DNA precipitation, all fractions were mixed with two volumes of PEG solution (30 % PEG 6000, 1.6 M NaCl) and 1 µL of nuclease free glycogen from mussels (Sigma-Aldrich, Germany), which had been tested for the absence of nucleic acids before via PCR. After 2 h of incubation at room temperature, samples were centrifuged for 30 min at maximum speed, supernatants were removed and pellets were washed with 150 µl of ice cold 70 % ethanol. Finally, pellets were resuspended in 30 µL of nuclease-free water. To ensure complete resuspension of the pellets, tubes were shaken at 1400 rpm on a mixing block (MB-102) (BIOER, China) for 1 min at 30 °C. Subsequently, the DNA concentration of all fractions was quantified using the QuantiFluor dsDNA System (Promega, Madison, USA) on a Qubit® 2.0 Fluorometer (ThermoFisher, USA).

Polymerase chain reactions (PCR) and DGGE of gradient fractions

For the comparison of the bacterial and fungal community composition in the individual SIP gradient fractions via DGGE, the bacterial 16S rRNA gene and the fungal ITS1 region were PCR amplified. The 50-µL PCR reaction contained 1x Herculase II reaction buffer, 50 mM $MgCl_2$, 250 µM dNTPs, 0.8 µg μL^{-1} BSA, 1 U Herculase Fusion DNA Polymerase (Agilent Technologies, Santa Clara, USA), 1 µL DNA template and 0.25 µM of the primers 341fgc and 907r (Muyzer et al., 1995) or ITS1fgc and ITS2 (Gardes and Bruns, 1993; White et al., 1990). The thermal cycling protocol consisted of an initial denaturation at 95°C for 2 min, denaturation at 95 C for 20 s, annealing at 54 °C for the 16S rRNA gene and 50 °C for the ITS1 region for 20 s, elongation at 72 °C for 20 s and final elongation at 72 °C for 3 min.

DGGE was performed using a DCode™ system (BioRad Laboratories GmbH; Munich, Germany). A 6.5 % polyacrylamide gel (acrylamide:bisacrylamide 37.5:1) with a denaturing gradient of formamide and urea ranging from 35 to 70 % for 16S rRNA gene amplicons and from 20 to 45 % for ITS1 amplicons was used. Electrophoresis was carried out in 0.5x TAE buffer (pH 7.4) for 16.5 h at 60 °C and 80 V (Muyzer et al., 1993). The gel was stained with 5 µl of GelGreen (1:10.000) in 50 mL of 1 x TAE buffer for 60 min and images were taken using the GelDoc^{TM} XR system (BioRad Laboratories GmbH; Munich, Germany).

Amplicon sequencing and DNA sequence analysis

For amplicon sequencing 16S rRNA genes were amplified using the primer set 515F and 806R, targeting a 291 bp product of the V4-V5 region of the 16S rRNA gene from *Archaea* and *Bacteria* (Bates et al., 2011). The fungal ITS1 region was amplified using the primer set ITS1F and ITS2 (Gardes and Bruns, 1993; White et al., 1990) to amplify a 180 bp product of the ITS1 region of fungi. Illumina amplicon sequencing was performed with one heavy and one light DNA-SIP fraction of each sample, as identified via DGGE. In each case, we selected the heavy and light fraction with the highest DNA concentration. This was always fraction 9 (light fraction) and 4 (heavy fraction). Amplicon sequencing and DNA sequence analysis were performed as described in Maarastawi et al. (2018). Only quality filtering was slightly modified by removing reads with an expected number of base call errors exceeding 0.3. Read files obtained after quality filtering were submitted to the European Nucleotide Archive (ENA) database under the project accession number PRJEB23769.

Identification of enriched taxa

To identify ^{13}C-labelled genera, the relative abundance of each genus in each analysed gradient fraction was used to calculate the enrichment factor (EF) according to Kramer *et al.* (2016):

EF = ^{13}C-heavy / ^{13}C-light – ^{12}C-heavy / ^{12}C-light.

Taxa that showed an EF ≥ 1 and a relative abundance of ≥ 0.1 % in the ^{13}C-labelled heavy fraction were considered as ^{13}C-labelled. Three further criteria were applied to ensure that taxa were indeed labelled, as postulated in Dallinger and Horn (2014): i) the relative abundance of a taxon in the ^{13}C-heavy fraction had to be higher than the relative abundance in the ^{13}C-light fraction, ii) the relative abundance of a taxon in the ^{13}C-heavy fraction had to be higher than the relative abundance in the ^{12}C-light fraction, III) the EF calculated for a taxon at day 0 had to be lower than the EF at the other sample time points.

Statistical analysis

Statistical analyses were conducted in R using the packages Vegan (Oksanen et al., 2008) and Phyloseq (McMurdie and Holmes, 2013). For all OTU-based analyses (i.e. Chao1, NMDS plots, ANOSIM), the original OTU table was filtered to contain sequences that were taxonomically classified as *Fungi*, or *Bacteria* and *Archaea.* OTUs that appeared with one or two reads in only one sample were discarded. For alpha-diversity estimation, evenly rarefied OTU tables were used. Chao1 indices were calculated and significant differences in bacterial and fungal alpha-diversity between the different incubation periods or compartments (bulk soil

versus rhizosphere at time point 21) were tested with non-parametric Kruskal-Wallis tests, as the data were non-normally distributed according to a Shapiro-Wilk test.

The microbial community composition was analysed by ordination of samples in NMDS plots based on Bray-Curtis dissimilarity matrices. For NMDS plots, the rarefied OTU tables were further pre-processed, so that sparse OTUs were removed. Therefore, a threshold of 0.3 was chosen, meaning that an OTU not found in at least 30 % of the samples was removed as statistically uninformative. To test for significant differences between groups of samples, ANOSIM was performed in Vegan with 999 permutations based on Bray-Curtis distances.

Differences between the EFs from different time points and between fungal and bacterial data were tested using a two-way analysis of similarity (ANOVA). A one-way ANOVA was used to further evaluate the differences between individual time points within one domain. Likewise, differences in the EFs of different compartments were evaluated based on one-way ANOVA. All ANOVAs were conducted after performing a Shapiro-Wilk test to check the data for normal distribution and Levene´s test to evaluate homogeneity of variances.

Results

Evaluation of successful ^{13}C-labelling of the bacterial and fungal community

In microcosm experiments, ^{13}C-labelled rice straw was added to soil from a paddy field undergoing rice-maize crop rotation. Bulk soil samples were retrieved after 3, 7, 14, 21, 28 and 35 days of incubation time, while rhizosphere samples were taken after 21 days. The incorporation of ^{13}C-carbon from labelled rice straw into the DNA of bacterial and fungal populations and the selection of representative heavy and light fractions for further detailed analyses after ultracentrifugation and gradient fractionation was done based on denaturing gradient gel electrophoresis using the 16S rRNA gene and ITS1 region as markers.

For all time points and compartments, distinct DGGE bands were observed in the heavy versus light fractions of the ^{13}C-samples. Such a shift was not observed in DGGE patterns of the corresponding ^{12}C-controls. A comparison of DGGE pattern between ^{13}C-heavy faction and ^{12}C-heavy fraction revealed an enrichment of specific bands in the ^{13}C-heavy fraction, confirming successful labelling (supplementary Figure 1). In order to identify the rice straw degrading bacterial and fungal taxa in the bulk soil samples and the rhizosphere of maize, amplicon sequencing was performed with a representative heavy and light fraction per ^{13}C-labelled and ^{12}C-labelled sample targeting the 16S rRNA gene and ITS1 region, respectively. After quality filtering and pre-processing, about 18.500 reads per fraction of 16S rRNA gene sequences and about 18.100 reads per fraction of the ITS1 region were used for further analyses.

Evaluation of the effect of time and compartment on straw degrading communities

Detailed analysis of the microbial community composition in samples treated with labelled straw indicated that the bacterial and fungal community composition of the active straw degrading microorganisms, detected in the ^{13}C-heavy fractions, changed significantly over time (bacteria: $R = 0.704$, $P < 0.001$; fungi: $R = 0.714$, $P < 0.001$), while this effect was minor in ^{13}C-light fractions (bacteria $R = 0.21$, $P < 0.05$; fungi $R = 0.103$, $P < 0.05$, Figure 1). In contrast, the samples of the ^{13}C-light fractions were mainly separated according to compartment (rhizosphere and bulk soil) in NMDS plots (bacteria: $R = 0.731$, $P < 0.001$; fungi: $R = 0.968$, $P < 0.001$; Figure 1).

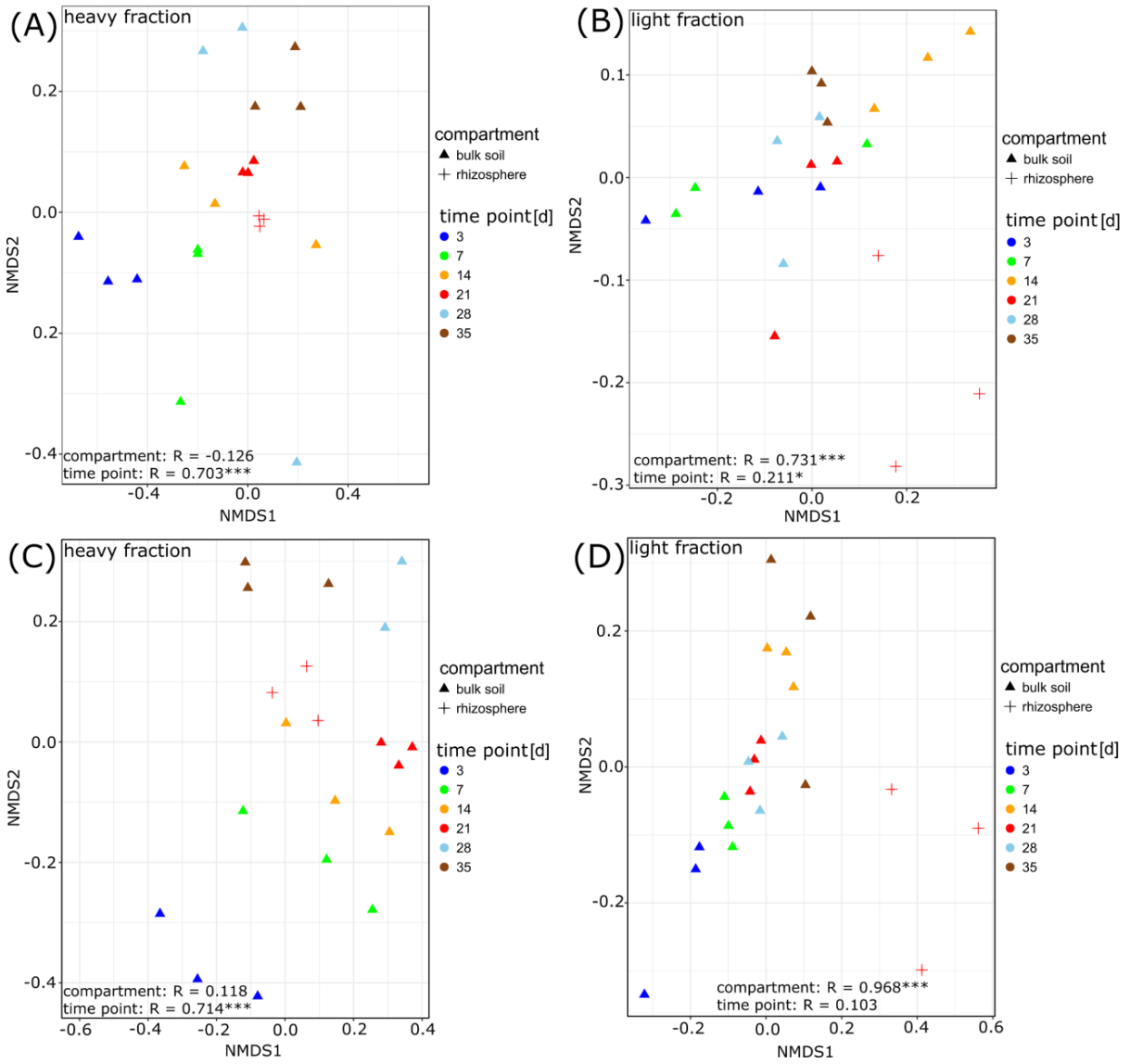


Figure 1: Ordination plots showing the influence of compartment and time on the active straw degrading (^{13}C-heavy fraction) and non-straw degrading (^{13}C-light fraction) bacterial (A, B) and fungal (C, D) community composition in ^{13}C-straw incubated samples. NMDS plots are based on Bray-Curtis dissimilarities, which were calculated from relative OTU abundances. Results of ANOSIM are shown with $P < 0.05$* and $P < 0.001$*** for the grouping factors.

As expected, the Chao1 richness was consistently lower in the ^{13}C-heavy fractions compared to the corresponding ^{13}C-light fractions for bacterial as well as for fungal communities. Noteworthy, the bacterial richness was higher than the fungal richness (Table 1). A comparison of the Chao1 richness of bulk soil and rhizosphere revealed that the bacterial richness in the rhizosphere of the ^{13}C-heavy fraction was slightly higher than in bulk soil ($P = 0.049$). The opposite, a slightly but not significant higher Chao1 richness in the bulk soil, was observed for fungal communities ($P = 0.126$). Furthermore, richness underwent a temporal dynamic in bacterial as well as in fungal communities in the ^{13}C-heavy fractions ($P = 0.036$ and $P = 0.018$, respectively; Table 1) with increasing richness in the ^{13}C-heavy fractions over time, before it decreased again. This was not evident in the ^{13}C-light fraction.

Table 1: Alpha-diversity of bacterial and fungal communities in heavy and light fractions of ^{13}C-straw incubated samples. Numbers represent mean values ± standard error of three biological replicates. Significant differences between samples collected at different time points or from different compartments were evaluated via Kruskal-Wallis tests.

Fraction	Sample	Time/Compartment	Bacteria		Fungi	
			Chao1	*P*-value	Chao1	*P*-value
^{13}C-heavy	Time point	3 days	711 ± 117	0.036	168 ± 5	0.018
		7 days	1134 ± 252		230 ± 40	
		14 days	939 ± 211		311 ± 148	
		21 days	1226 ± 50		300 ± 22	
		28 days	1460 ± 297		173 ± 65	
		35 days	1189 ± 140		234 ± 39	
	Compartment	bulk soil	1226 ± 50	0.049	300 ± 22	0.126
		rhizosphere	1303 ± 173		237 ± 13	
^{13}C-light	Time point	3 days	1878 ± 201	0.795	396 ± 30	0.098
		7 days	1780 ± 129		446 ± 61	
		14 days	1769 ± 158		501 ± 81	
		21 days	1912 ± 401		554 ± 116	
		28 days	1800 ± 122		428 ± 57	
		35 days	1760 ± 191		347 ± 45	
	Compartment	bulk soil	1912 ± 401	0.275	554 ± 116	0.049
		rhizosphere	1717 ± 275		239 ± 65	

Identification of the straw degrading microorganisms based on enrichment factor analysis

To identify the active straw degrading microbial taxa, the EF was calculated according to Kramer *et al.* (2016) for each genus based on read abundances in all four analysed fractions (^{12}C-heavy, ^{12}C-light, ^{13}C-heavy and ^{13}C-light). The EF reflects the ratio between labelled and unlabelled members of a specific taxon. The higher the EF of a taxon, the more members of its population were labelled and retrieved from the heavy fraction of the SIP gradient. We applied very stringent conditions (EF $\geq$ 1; relative read abundance in the ^{13}C-heavy fraction $\geq$ 0.1 %) for the identification of labelled genera, focussing on those with a notable DNA enrichment in the ^{13}C-heavy fraction. Three further conditions had to be fulfilled to consider a genus as "labelled", which were postulated before (Dallinger and Horn, 2014). Applying these conditions, about 25 % (109 of 438) of all detected bacterial genera and 9 % (17 of 185) of the fungal genera were considered as labelled and were included in the following analyses (a complete list of labelled genera is provided in supplementary Tables 1 and 2).

Overall, the EFs of bacteria versus fungi (including all time points and labelled genera) were not significantly different, indicating no major differences in the intensity of label incorporation between these groups. However, we detected significant differences over time in both groups, especially for bacteria (ANOVA for bacteria: $P < 0.05$; Figure 2). After 3 days of incubation, we observed a stronger increase in bacterial EFs compared to fungal EFs. The EFs of bacteria remained high during the first two weeks of incubation before they declined, whereas the EFs of fungi increased to maximum levels after three to four weeks of incubation.

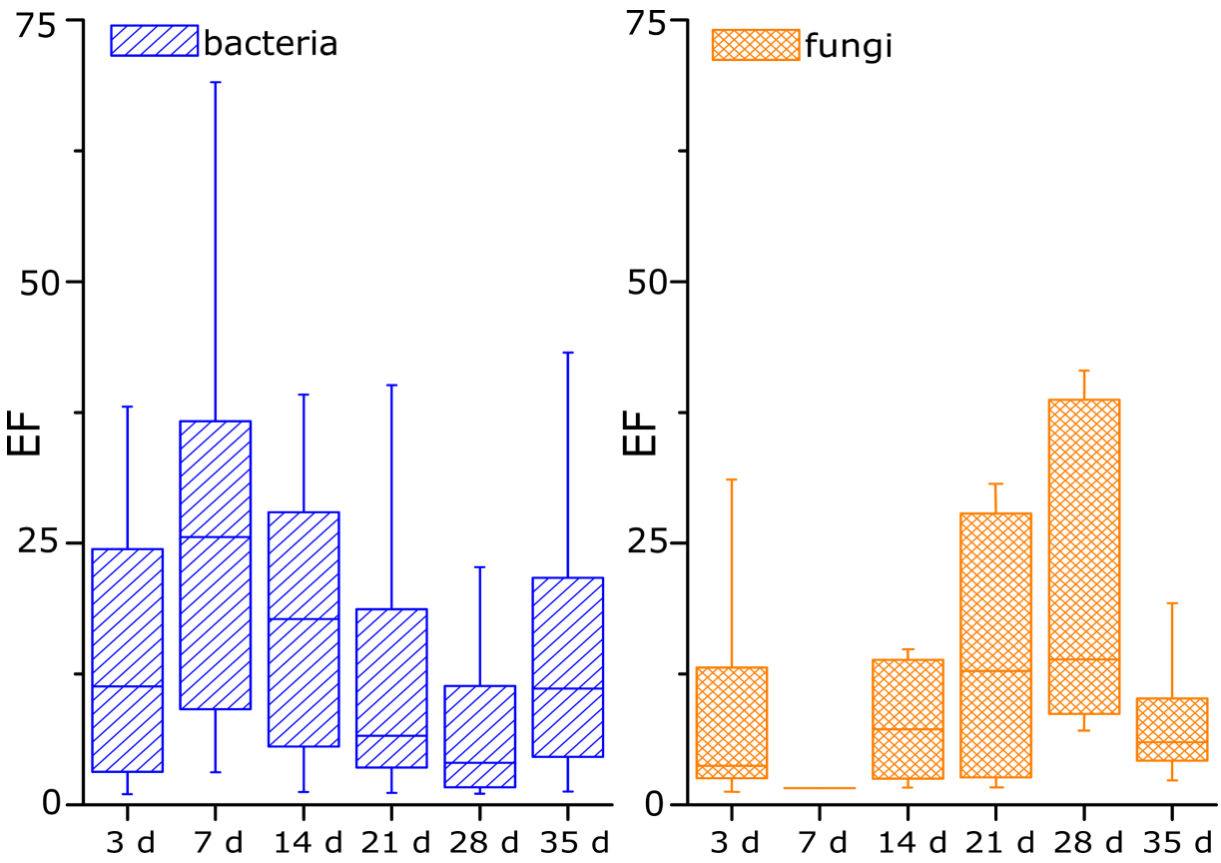


Figure 2: Variation of EFs of ^{13}C-labelled bacterial (blue) and fungal (orange) genera over time. The box plot shows the median values, 25th and 75th percentile and the standard error.

Identity of the straw degrading microorganisms and their temporal dynamics in bulk soil

Over time, the number of bacterial classes with labelled genera increased from 7 to 14. Most of the ^{13}C-incorporating bacterial genera belonged to the *Alpha-, Beta-, Gammaproteobacteria, Actinobacteria* and *Bacilli* (Figure 3 A and B). The relative abundance of labelled *Bacilli* increased over time (up to 58 %), especially after the first week of incubation (Figure 3 A). Remarkably, the number of labelled genera within the class *Bacilli* remained quite stable (Figure 3 B), indicating that only a few genera of *Bacilli* formed a large population of labelled organisms over time. Labelled genera of *Actinobacteria* were low abundant at the beginning, but increased consistently in relative abundance (up to 15 %) (Figure 3 A) and with regard to diversity (Figure 3 B) over time. In contrast, the relative abundance of labelled *Gammaproteobacteria* decreased substantially from approx. 32 % to less than 2 % between 7 and 14 days of incubation. A similar shift was observed for the *Betaproteobacteria*, while the relative abundance and diversity of labelled *Alphaproteobacteria* remained quite stable over time.

The temporal dynamics were evaluated in more detail at genus level (Figure 3 C). This revealed that the prominent labelling of *Bacilli* was due to three abundant (> 1 %) genera, i. e. *Bacillus,*

Paenibacillus and *Cohnella* (Figure 3 C). *Paenibacillus* and *Cohnella* were detected at all time points, while *Bacillus* was detected as labelled at day 14 for the first time. Overall, 20 % of all labelled genera were detected at most or all time points. Some of them were additionally highly abundant, e. g. *Rhizobium*, *Paenibacillus* and *Cohnella*. *Rhizobium* and *Cohnella* were the only taxa that showed high relative abundances as well as high EFs (EF > 5) and contributed thus substantially to straw metabolisation. Other genera that were strongly labelled and detected at most time points include *Arthrobacter*, *Asticcacaulis*, *Caulobacter*, *Devosia*, *Microvirga* and *Pseudoxanthomonas,* but these had lower relative abundances (Figure 3 C). Most of these genera represent *Alphaproteobacteria*. Although this class had a lower relative abundance, it had a higher diversity of labelled genera compared to the *Bacilli* (Figure 3 A, B). Genera that were only labelled at the beginning of the experiment had often high EFs but low relative abundances (e. g. *Brevundimonas*, *Sphingobium* and *Simplicispira*). Only *Cellvibrio* and *Pseudoduguanella* were strongly labelled and highly abundant at the beginning of the experiment. Genera that appeared after 14, 21 or 28 days (e. g. *Agromyces*, *Aquicella*, *Arenimonas*, *Burkholderia*, *Dynella*, *Gemmata*, *Haliangium*, *Kribbella*) were all of rather low relative abundance.

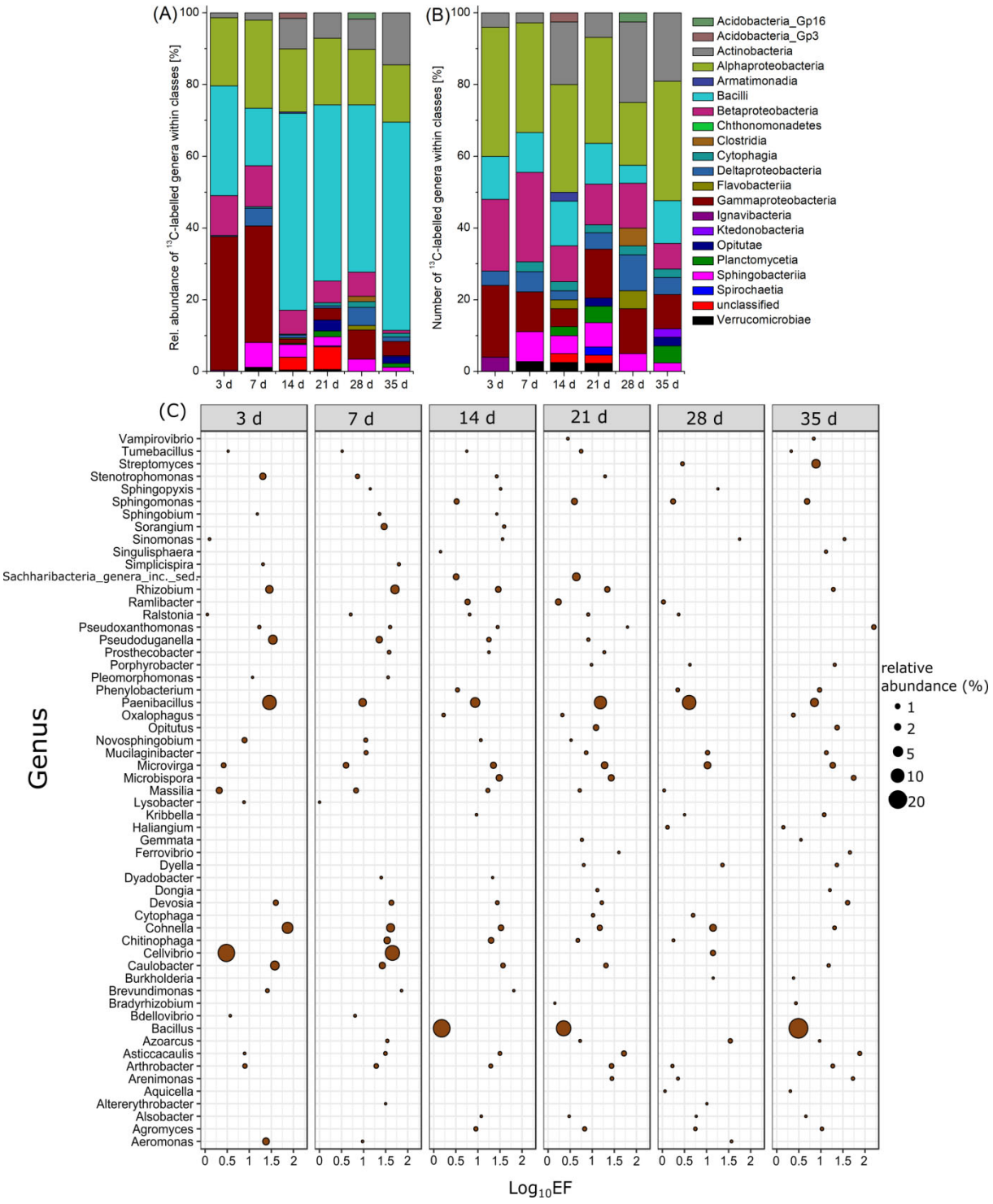


Figure 3: ^{13}C-labelled bacterial classes (A, B) and genera (C) identified after 3, 7, 14, 21, 28 and 35 days of incubation with ^{13}C-labelled rice straw in bulk soil samples. For each class, the labelled genera identified in the ^{13}C-heavy fraction belonging to one class were counted (B) or their relative abundances were summed up (A). The EFs of the labelled genera are presented in (C) as $\log_{10}$ transformed values on the x-axis, while the relative abundance of each genus in the ^{13}C-heavy fraction is displayed via bubble size. For reasons of clarity, we excluded bacterial genera that were identified as labelled at only one time point from (C). The complete dataset is available in supplementary Table 1.

The diversity of labelled fungal taxa was much lower and limited to six classes. Most of them belonged to the classes *Sordariomycetes* and *Agaricomycetes*, which were detected consistently at nearly all time points and with high relative abundances (Figure 4 A, B). The detection of members of the other classes was limited to a few or only one time point. Strongly involved in straw metabolisation were *Thielavia* as well as *Piriformospora*, members of the classes *Sordariomycetes* and *Agaricomycetes,* respectively, which were detected at almost every time point and with high relative abundance. Further strongly labelled genera (EF > 10) that belonged to the class *Sordariomycetes* were *Zopfiella*, *Myrothecium* and *Conlarium* (Figure 4 C). The highest number of labelled fungal genera was detected after three days of incubation (9 genera, representing 5 classes). Several of these genera were not detected at later time points, even some with high EFs or high relative abundance at the early time point (*Malassezia*, *Conlarium*, *Fusarium*). At the later time points (14 days or later), other genera including *Myrothecium* and *Cladorhinum* appeared.

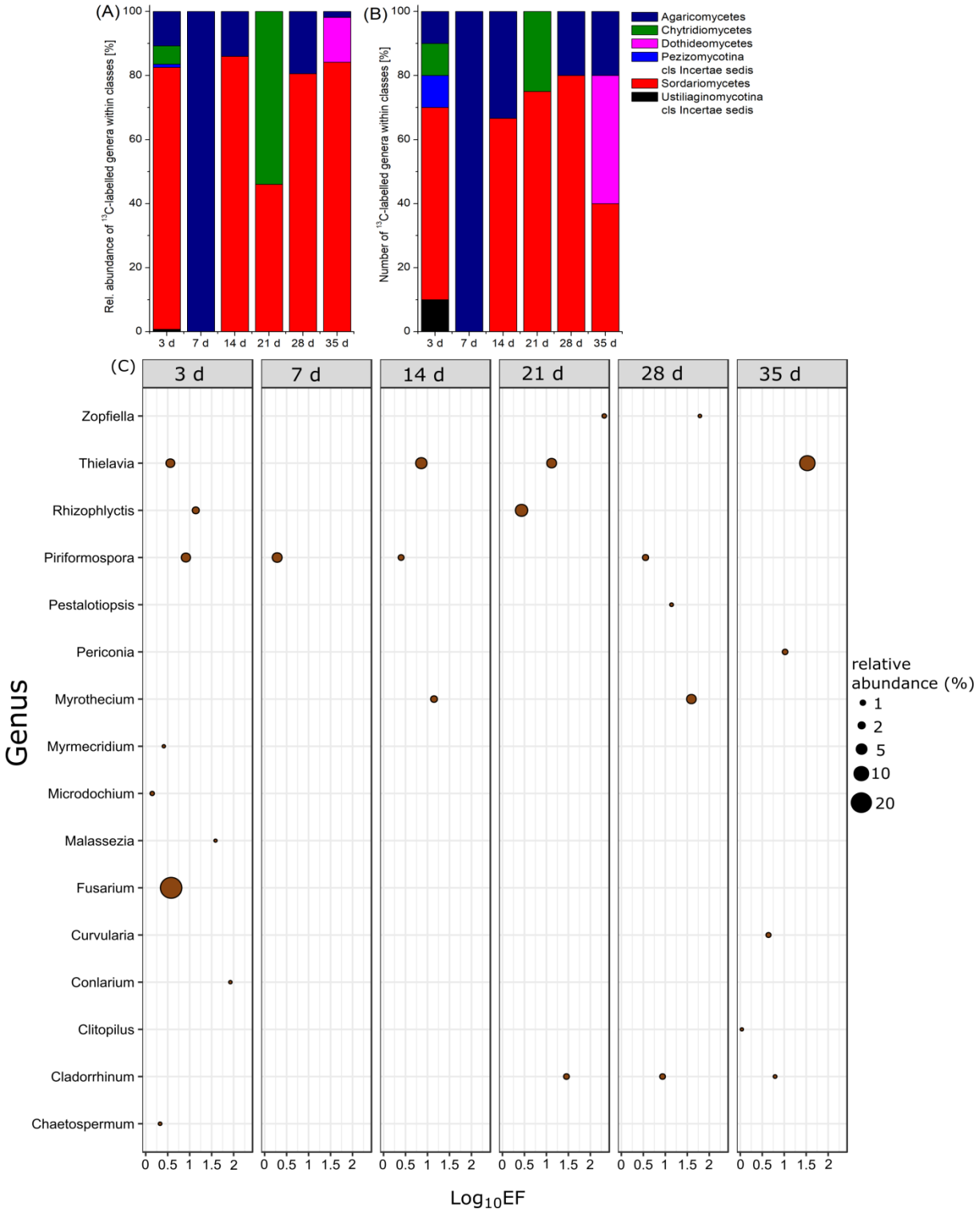


Figure 4: ^{13}C-labelled fungal classes (A, B) and genera (C) identified after 3, 7, 14, 21, 28 and 35 days of incubation with ^{13}C-labelled rice straw in bulk soil samples. For each class the labelled genera identified in the ^{13}C-heavy fraction belonging to one class were counted (B) or their relative abundances were summed up (A). The EFs of the labelled genera are presented in (C) as $\log_{10}$ transformed values on the x-axis, while the relative abundance of each genus in the ^{13}C-heavy fraction is displayed via bubble size.

Comparison of labelled microorganisms in bulk soil and rhizosphere

To evaluate differences in the straw metabolising communities between the rhizosphere and the bulk soil, we focussed on a comparison of the rhizosphere sample with the bulk soil sample taken at the same day, i. e. day 21. Other time points were excluded due to the temporal shift in the labelled microbial community in the bulk soil. In total, we identified 41 labelled bacterial genera in the rhizosphere, 20 of these were labelled in the bulk soil as well (Figure 5, Table 2).

Table 2: Identity of bacterial and fungal genera that were exclusively labelled in the rhizosphere after 21 days of incubation. Genera that were uniquely labelled in the rhizosphere even when compared to all bulk soil samples are highlighted in bold. Relative abundance was calculated in relation to all genera identified in the ^{13}C heavy fraction.

Bacterial genera	**Rhizosphere EF**	**Rhizosphere relative abundance (%)**
Altererythrobacter	10.47	0.11
Alterococcus	3.57	0.12
Candidatus_Solibacter	1.31	0.12
Cystobacter	5.29	0.34
Gemmatimonas	1.81	0.77
Gp3	2.95	0.61
Haliangium	6.96	1.07
Kribbella	6.42	0.19
Labilithrix	2.28	0.13
Lysobacter	11.33	0.13
Marmoricola	7.87	0.45
Mesorhizobium	6.85	0.13
Microbacterium	5.28	0.11
Micromonospora	7.16	0.64
Unclassified Caldilineaceae	1.00	0.28
Nocardioides	6.83	0.19
Pseudolabrys	20.18	0.11
Roseomonas	3.58	1.50
Sorangium	10.33	1.81
Zavarzinella	1.24	0.10
Zoogloea	7.93	0.14
Fungal genera		
Ascobolus	14.40	0.22
Conlarium	122.25	0.25
Piriformospora	26.66	1.08

Although the number of labelled fungal genera was nearly 10-fold lower, the degree of overlap with the bulk soil compartment was roughly comparable.

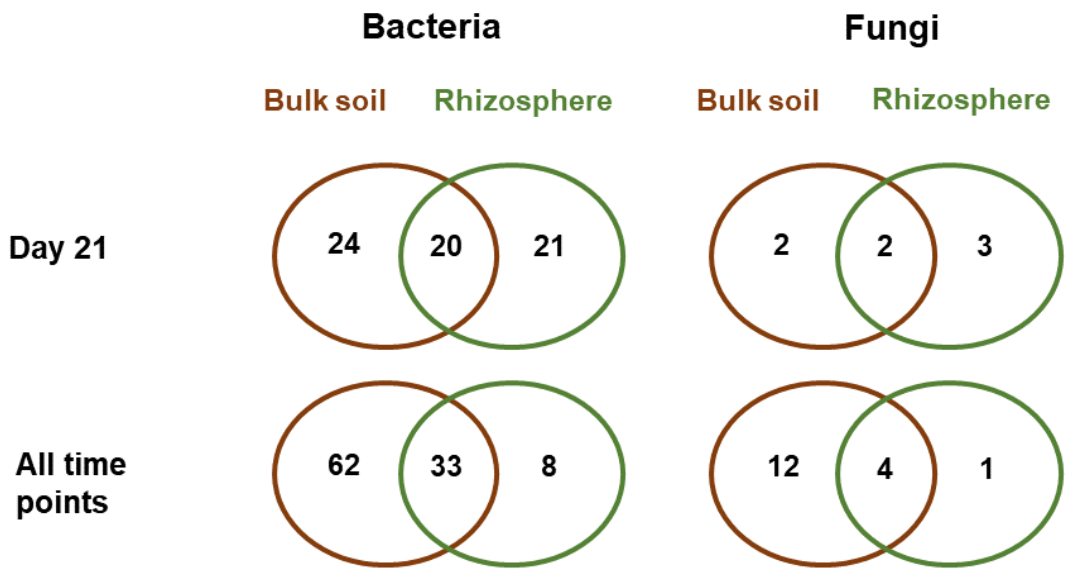


Figure 5: Venn diagrams displaying numbers of compartment-specific and -independently labelled bacterial and fungal genera. The upper Venn diagrams show the number of labelled genera after 21 days of incubation with ^{13}C-labelled straw. The lower diagrams show a more stringent comparison including all time points (3, 7, 14, 21, 28 and 35 days) for the bulk soil samples.

The overall label intensity of all labelled bacterial taxa was significantly higher ($P < 0.05$) in the bulk soil with an average EF of 15 than in the rhizosphere (average EF of 6) (Figure 6 A). Among the bacterial genera that were detected in both compartments, *Planctopirus* was the genus with the strongest labelling, but the EF was four times higher in bulk soil (EF = 120) than in the rhizosphere (EF = 30). Fourteen further genera, which were labelled in both compartments, were more strongly labelled in the bulk soil than in the rhizosphere, whereas six genera had higher EFs in the rhizosphere (*Agromyces*, *Bacillus*, *Bradyrhizobium*, *Gemmata*, *Sphingomonas*, *Vampirovibrio*). Especially *Badyrhizobium* was labelled threefold more strongly in the rhizosphere. Likewise, *Altererythrobacter*, *Lysobacter*, *Pseudolabrys* and *Sorangium* showed a stronger labelling in the rhizosphere (EFs > 8). Only five genera of fungi were labelled after 21 days of incubation in the rhizosphere, with *Thielavia* and *Zopfiella* being labelled in both compartments (Figure 6 B). The label intensity and relative abundance of these genera was slightly higher in the bulk soil. The genera *Ascobolus*, *Conlarium* and *Piriformospora* were identified as exclusively labelled in the rhizosphere at day 21 (Table 2).

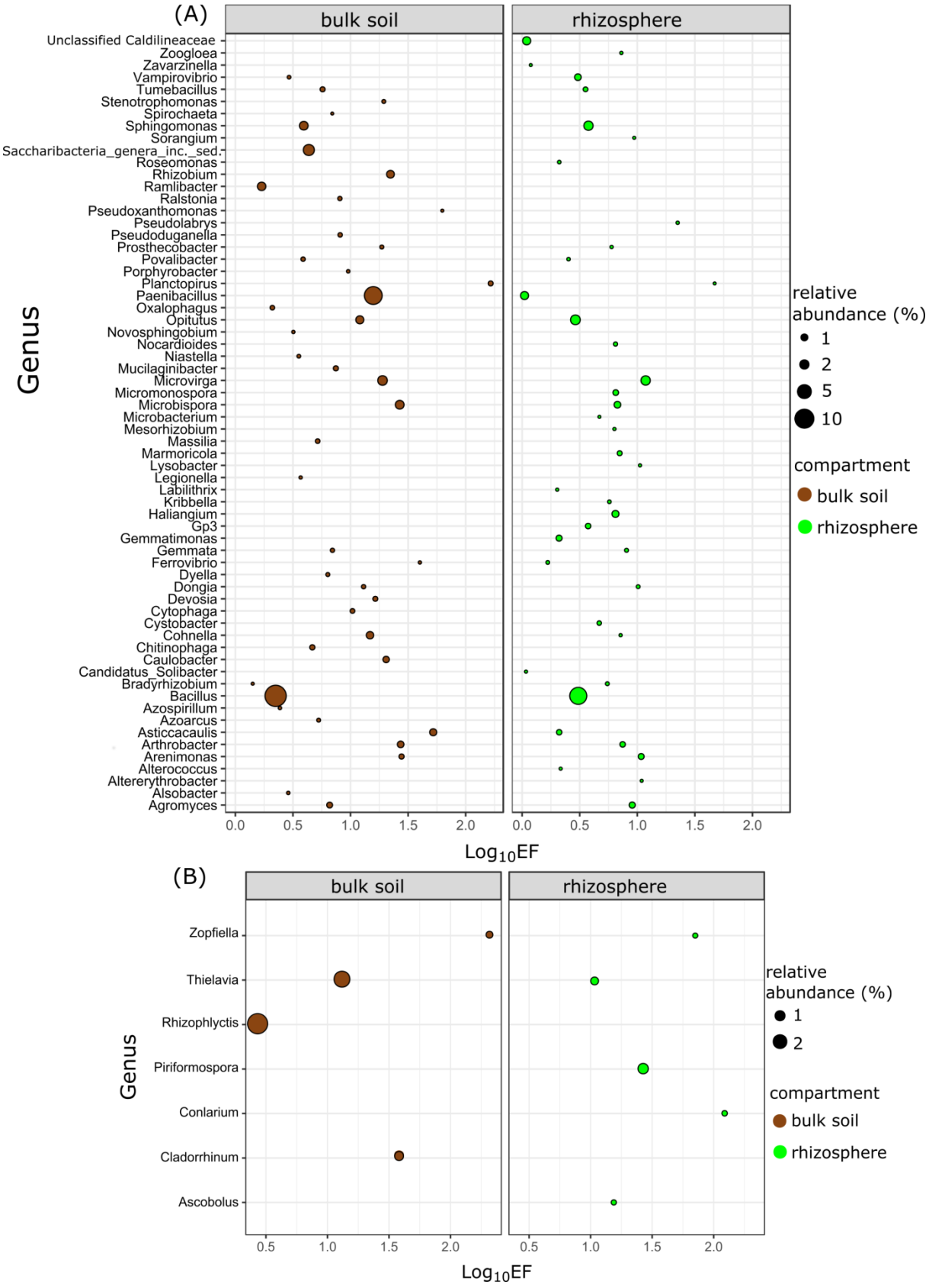


Figure 6: ^{13}C-labelled bacterial (A) and fungal (B) genera identified in rhizosphere and bulk soil samples after 21 days of incubation. The EFs of labelled taxa are presented as $\log_{10}$ transformed values on the x-axis, while the relative abundance of the ^{13}C-heavy fraction of each genus is displayed via bubble size.

A more stringent comparison including all bulk soil sampling time points revealed that eight bacterial genera and one fungal genus remained uniquely labelled in the rhizosphere (Figure 5, Table 2). Focussing specifically on those taxa that were identified as labelled in the rhizosphere, the comparison of EFs between compartments revealed that 46 % of all bacterial genera and 60 % of all fungal genera showed higher EFs in the rhizosphere (supplementary Figures 2 and 3), pointing to a stronger involvement in straw metabolisation of these genera in the rhizosphere than in bulk soil. Remarkably, bacterial and fungal taxa exclusively labelled in the rhizosphere were not even detected in the unlabelled fraction of the bulk soil communities at the different days (i. e. in the ^{13}C-light as well as in the ^{12}C-heavy and ^{12}C -light fractions), indicating that they became specifically enriched in the rhizosphere.

Discussion

Temporal succession of the rice straw degradation process in bulk soil

We investigated the rice straw degradation process in a paddy soil under rice-maize crop rotation during the period of maize cultivation by DNA-SIP. Analysis of the ^{13}C-labelled bacterial and fungal genera over time showed that the straw degrading microbial community underwent a strong temporal dynamic with changes in composition as well as in richness. This is in good agreement with previous studies reporting a temporal dynamic of the degradation of FOM, like plant residue or cellulose (Bernard et al., 2007; Haichar et al., 2007; Lee et al., 2017). The richness of the bacterial and fungal straw degraders increased during the first weeks of incubation, before it declined again slightly (Table1). In contrast, the richness of the unlabelled communities (light fraction) was constant over time, indicating that only the straw degrading community underwent this dynamic. The lower richness of the active straw degrading microorganisms (Table 1) indicates that only a few soil inhabiting taxa profited from the FOM as carbon source, as described in Bastian et al. (2009) for the degradation of wheat straw in oxic soils. Actually, approximately 25 % of all detected bacterial and 9 % of all detected fungal genera were identified to be involved in straw degradation.

The labelling of bacterial taxa was already strong at the beginning of the experiment after three days of incubation, whereas fungal taxa were more strongly labelled at later time points of the experiment, i. e. after 21 days (Figure 2), indicating that the addition of FOM leads to differential growth responses of bacterial and fungal genera. These differential responses can be related to changes in the availability of different carbon compounds during the degradation process (Bastian et al., 2009). The straw degradation process has been described to be induced by copiotrophic, fast growing bacteria. Such taxa show a strong label incorporation at the

beginning of the experiment and take advantage of the readily degradable carbon compounds in the FOM (Pascault et al., 2013). Members of the classes *Alpha-, Beta-, Gammaproteobacteria* (e. g. *Rhizobium*, *Pseudoduganella*, *Cellvibrio*), which we identified in our study as early straw degraders, were also previously proposed to be copiotrophic (Bernard et al., 2007; Fierer et al., 2007) (Figure 3). In contrast, the fungal community was less diverse at the beginning of the experiment with only a few taxa being labelled, with the exception of day 3. Over time, further bacterial and fungal taxa became involved in the straw degradation process (Figure 2). Several of them are known as oligotrophs e.g. bacteria belonging to *Actinobacteria* (*Agromyces*, *Kribella*) or *Deltaproteobacteria* (*Haliangium*) and fungal taxa like *Zopfiella, Pestalotiopsis, Myrothecium* and *Cladorrhinum* (Bastian et al., 2009; Bernard et al., 2007; Fierer et al., 2007), The delayed label incorporation by *Actinobacteria* as well as by the fungal community (Figure 2) may thus result from a more oligotrophic life style, probably linked to the capability to decompose more complex carbon compounds, which might not be degraded by the early degrading bacterial taxa (Fan et al., 2014; Panikov, 1995; Tardy et al., 2015). This indicates that more difficult to degrade carbon compounds may have served as substrates after 2 – 3 weeks of straw addition. An additional possible explanation for the later stimulation of some fungal taxa might be the bacterial production of growth factors essentially needed for fungal growth, as proposed for some vitamins (De Boer et al., 2005). Remarkably, the bacterial activity increased a second time (Figure 2) at the latest sampling date. It thus followed the increased fungal activity after 21 days. Fungal activity may have led to an additional release of straw breakdown products, potentially stimulating the bacterial population again (De Boer et al., 2005). Moreover, the second increase in bacterial activity may have occurred due to cross-feeding of carbon compounds released by the fungi or the availability of labelled dead biomass of primary degraders (Uhlík et al., 2009).

In summary, the analysis of the straw degradation process in bulk soil at high temporal resolution enabled us to identify different phases of bacterial and fungal activity. The shifts in richness of labelled taxa over time corresponded well to the activity of different microbial groups that became involved in the straw degradation process over time: i) copiotrophic organisms in the initial phase ii) oligotrophic fungi and bacteria after two to three weeks, and iii) microorganisms that used breakdown products of fungi or cross feeders at the latest time points.

Identification of active straw degrading microorganisms in paddy soil under crop rotation

Most of the labelled bacterial genera belonged to the classes *Alpha-, Beta-* and *Gammaproteobacteria* (Figure 3) and about 90% of these genera are known to be obligate aerobic. The mentioned classes were previously identified as degraders of plant residue in different oxic soils (Bastian et al., 2009; Bernard et al., 2007; Cleveland et al., 2007). Further taxa like *Actinobacteria* (e.g. *Arthobacter*) and *Bacilli* (e.g. *Bacillus*) were also previously detected in different oxic soils as degraders of fresh plant residue. Both genera, *Arthrobacter* and *Bacillus*, are known to be able to synthesize extracellular enzymes to degrade complex carbon compounds (Fan et al., 2014; Mau et al., 2015; Panikov, 1995; Zhao et al., 2016). Anaerobic bacteria that are known to be involved in rice straw degradation in paddy soils under anoxic conditions such as *Clostridia* or methanogens like *Methanosarcina* (Conrad et al., 2012; Lee et al., 2012) were not among the labelled organisms in our study. This suggests that straw degradation occurs by a distinct microbial community in paddy soil when fields are periodically managed under non-flooded conditions. The absence of labelled methanogens is remarkable, because Shrestha et al. (2011) and Lee et al. (2012) identified an increased relative abundance of methanogens upon residue addition in paddy soils even when soil was incubated under oxic conditions. In contrast, our data show that methanogens do not profit from rice straw carbon when rice is incorporated during the phase of maize cultivation. The absence of metabolically active methanogens profiting from rice straw indicates that decreased methane emissions can be expected upon rice straw application, when this is done in a field managed under crop rotation.

The low number of labelled fungal taxa (Table 1, Figure 5) suggests that fungi are less important for the degradation of straw in paddy soils undergoing rice-maize crop rotation, even though their EFs are comparable to those of bacteria. From paddy soils under rice monoculture, it is known that fungi are of little relevance for the straw degradation process (Nakamura et al., 2003). It is assumed that the flooded and thus anoxic conditions inhibit the colonization by fungi (Lumini et al., 2011; Murase et al., 2006; Nakamura et al., 2003). The periodically oxic conditions during rice-maize crop rotation may have supported the growth of some fungal genera. Most of the labelled fungal genera in our study belong to the classes *Sordariomycetes* (*Zopfiella*, *Cladorrhinum*, *Pestalotiopsis*) and *Agaricomycetes* (*Piriformospora*) (Figure 4). *Piriformospora* was highly abundant and appeared at almost all time points. Moreover, it was strongly labelled in both compartments, bulk soil and rhizosphere. Members of this genus have a saprophytic live style (Bokati and Craven, 2016), are known to occur in mutualistic association with plants such as maize (Qiang et al., 2012) and were detected in maize field soil

and paddy soil before (Maarastawi et al., 2018). *Sordariomycetes* are known to be the key players in organic residue decomposition in agricultural soils (España et al., 2011; Kjøller and Struwe, 2002; Murase et al., 2012; Niu et al., 2015). Some of the labelled genera of this class are often described as plant endophytes e.g. *Cladorrhinum* and *Pestalotiopsis* (Gasoni and Stegman de Gurfinkel, 1997; Strobel et al., 1996) or are known from aquatic ecosystems like *Zopfiella* (Daferner et al., 2002), which may explain the occurrence in rice paddies, which undergo periodic flooding.

In conclusion, the straw degrading microbial community was found to represent a typical straw degrading community as known from oxic soils, though with little diversity concerning the fungal players. Anaerobic microorganisms did not respond to straw application. This is remarkable because our previous study showed that rice-maize crop rotation soils host bacterial communities that are more similar to those of flooded soils than to those of non-flooded soils (Maarastawi et al., 2018), and an involvement of anaerobic microorganisms in straw degradation even under oxic incubation conditions has been reported for paddy soils (Lee et al., 2012).

Differences between straw degrading communities in bulk soil and rhizosphere

Even though the rhizosphere microbiota is known to live from root released carbon compounds, it has been shown that microbes residing in the rice rhizosphere profit from straw derived carbon compounds in paddy soils (Shrestha *et al.,* 2011). However, it remained unknown whether the straw-degrading taxa in the rhizosphere are the same as in bulk soil or whether different microorganisms are profiting from rice straw in the rhizosphere. To address this question, we directly compared the straw degrading microbial community in the bulk soil and rhizosphere. Overall, labelled bacterial taxa in the bulk soil had higher EFs than in the rhizosphere, while it was almost the same label intensity for the fungal taxa. This indicates less intensive straw utilization in the rhizosphere, where microorganisms encounter a continuous carbon input via rhizodeposition processes (Kuzyakov, 2010; Lu et al., 2004). Further, we observed that some taxa were specifically labelled in the rhizosphere but not in bulk soil (Table 2), indicating that a specialized community benefits from straw derived carbon in the rhizosphere. Many of the taxa we identified as straw degraders exclusively in the rhizosphere are known to inhabit the rhizosphere of plants, e. g. *Zoogloea*, *Roseomonas*, *Zavarzinella, Microbacterium, Lysobacter* and *Mesorhizobium* (Chung et al., 2015; Jilani et al., 2007; Naz et al., 2014; Smalla et al., 2001; Yang and Crowley, 2000) (Figure 6), suggesting that these organisms are able to use root exudates as well as carbon derived from additional resources. *Lysobacter* and *Mesorhizobium* were described before as inhabitants of the maize rhizosphere

with strong lytic abilities to degrade macromolecules (Fan et al., 2014; Postma et al., 2011; Sanguin et al., 2006). We also identified organisms labelled in both compartments but with higher relative abundances and EF in the ^{13}C-heavy fraction of the rhizosphere sample like *Vampirovibrio* and *Sphingomonas*. This indicates that these taxa decompose straw preferentially in the rhizosphere, possibly due to the additional supply of root exudates or better life circumstances. Interestingly, *Vampirovibrio* is known as predatory cyanobacterium (Soo et al., 2015). Thus, it may be a cross feeder, profiting from primary straw-degraders. This is further substantiated by the fact that *Vampirovibrio* is found strongly labelled at later time points.

Likewise as in bulk soil, we found a lower number of labelled fungal than bacterial genera in the rhizosphere (Figure 6). The genera *Piriformospora*, *Conlarium* and *Ascobolus*, which were identified as specifically labelled in the rhizosphere, were previously detected as rhizosphere organisms of maize and as inhabitants of paddy soil (Maarastawi et al., 2018; Qiang et al., 2012), as rhizosphere organisms of *Huperzia serrata* (Wang et al., 2016) or as rhizosphere organisms of tobacco (Li *et al.*, 2017; Li *et al.*, 2017b), respectively. One genus of arbuscular mycorrhizal (AM) fungi was present in the rhizosphere (*Entrophospora*), but was not labelled, indicating that AM fungi use mainly root derived carbon.

The detection of specially labelled microbial taxa in the rhizosphere suggests that a subpopulation of the rhizosphere microbiota benefits from straw addition. As several of these taxa were not detected in the bulk soil, even not in the unlabelled fractions, it appears that these microorganisms use both, root released carbon as well as organic carbon from the straw residues. Alternatively or additionally, their specific growth in the rhizosphere might have been supported by other soil physicochemical or biological parameters that are different in the rhizosphere compared to the bulk soil, e. g. soil pH or availability of specific substances such as vitamins, needed for growth. Interestingly, some of the labelled bacterial and several fungal genera that were labelled in the rhizosphere are known to have a rather oligotrophic lifestyle and are known to be able to grow on recalcitrant carbon compounds, e.g. *Roseomonas* or *Zoogloea* (Bais et al., 2006; Furuhata et al., 2008; Zhang and Zhou, 2016). The presence of straw as additional carbon source may have supported the development of these genera in the rhizosphere.

Conclusion

Straw degradation in paddy soil is a highly dynamic process undergoing a temporal succession. Some bacterial taxa began immediately with the decomposition of readily degradable straw compounds, while fungal genera were most active at later stages, probably profiting from more complex carbon compounds. Only a small subpopulation of the whole bacterial and fungal community became labelled, indicating that only a small part of the soil inhabiting taxa used the straw as carbon source. The most prominent straw degraders were aerobic bacterial taxa belonging to *Alpha*-, *Beta*-, *Gammaproteobacteria* and *Bacilli* as well as fungal taxa belonging to *Sordariomycetes* and *Agaricomycetes*, i. e. taxa that were shown to be involved in straw degradation in oxic soils. In contrast, anaerobic taxa, which are known to be involved in rice straw degradation in paddy soil under flooded conditions, but even in paddy soil incubated under oxic conditions, were not labelled. Thus, straw degradation in paddy soil undergoing crop rotation appears to be an aerobic process, even though the total microbial community in these soils remains similar to that of rice monoculture soils, characterised by the presence of diverse anaerobic microorganisms (Breidenbach et al., 2016; Maarastawi et al., 2018). Crop rotation management will therefore contribute to a reduction of methane emissions when rice straw is returned to the soil. Whether the rice straw degradation process is fully aerobic from the beginning on even under field conditions remains to be evaluated. Straw is not only metabolized by microorganisms in the bulk soil, but also in the maize rhizosphere. Our study clearly showed that microorganisms specifically enriched in the rhizosphere do profit from this additional carbon source. In general, label intensity was lower in the rhizosphere, even though some genera were uniquely or more strongly labelled in the rhizosphere compared to bulk soil. This indicates that rhizosphere-inhabiting microorganisms are less dependent on straw derived carbon than those in the bulk soil.

Acknowledgements

We are thankful to the International Rice Research Institute (IRRI) and especially to Rainer Wassmann for providing research space at IRRI. The Max Planck-Genome-centre Cologne (http://mpgc.mpipz.mpg.de/home/) is acknowledged for performing Illumina sequencing. Moreover, we thank Judith Harz and Merle Noschinski-Reetz for excellent technical assistance.

References

Asari, N., Ishihara, R., Nakajima, Y., Kimura, M., Asakawa, S., 2007. Succession and phylogenetic composition of eubacterial communities in rice straw during decomposition on the surface of paddy field soil. Soil Science and Plant Nutrition 53, 56–65. doi:10.1111/j.1747-0765.2007.00110.x

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–266. doi:10.1146/annurev.arplant.57.032905.105159

Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biology and Biochemistry 41, 262–275. doi:10.1016/j.soilbio.2008.10.024

Bates, S.T., Berg-lyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. Examining the global distribution of dominant archaeal populations in soil. The ISME Journal 5, 908–917. doi:10.1038/ismej.2010.171

Bernard, L., Mougel, C., Maron, P.-A., Nowak, V., Lévêque, J., Henault, C., Haichar, F. el Z., Berge, O., Marol, C., Balesdent, J., Gibiat, F., Lemanceau, P., Ranjard, L., 2007. Dynamics and identification of soil microbial populations actively assimilating carbon from ^{13}C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. Environmental Microbiology 9, 752–764. doi:10.1111/j.1462-2920.2006.01197.x

Bokati, D., Craven, K.D., 2016. The cryptic Sebacinales: An obscure but ubiquitous group of root symbionts comes to light. Fungal Ecology 22, 115–119. doi:10.1016/j.funeco.2016.01.010

Breidenbach, B., Blaser, M.B., Klose, M., Conrad, R., 2016. Crop rotation of flooded rice with upland maize impacts the resident and active methanogenic microbial community. Environmental Microbiology 18, 2868–2885. doi:10.1111/1462-2920.13041

Cabangon, R.J., Tuong, T.P., 2000. Management of cracked soils for water saving during land preparation for rice cultivation. Soil and Tillage Research 56, 105–116. doi:10.1016/S0167-1987(00)00125-2

Chung, E.J., Yoon, H.S., Kim, K.H., Jeon, C.O., Chung, Y.R., 2015. *Roseomonas oryzicola sp.* Nov., isolated from the rhizosphere of rice (*Oryza sativa l.*). International Journal of Systematic and Evolutionary Microbiology 65, 4839–4844. doi:10.1099/ijsem.0.000656

Cleveland, C.C., Nemergut, D.R., Schmidt, S.K., Townsend, A.R., 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82, 229–240. doi:10.1007/s10533-006-9065-z

Conrad, R., Klose, M., Lu, Y., Chidthaisong, A., 2012. Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. Frontiers in Microbiology 3, 1–12. doi:10.3389/fmicb.2012.00004

Daferner, M., Anke, T., Sterner, O., 2002. Zopfiellamides A and B, antimicrobial pyrrolidinone derivatives from the marine fungus *Zopfiella latipes*. Tetrahedron 58, 7781–7784. doi:10.1016/S0040-4020(02)00942-0

Dallinger, A., Horn, M.A., 2014. Agricultural soil and drilosphere as reservoirs of new and unusual assimilators of 2,4-dichlorophenol carbon. Environmental Microbiology 16, 84–100. doi:10.1111/1462-2920.12209

De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: Impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811. doi:10.1016/j.femsre.2004.11.005

España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G.D., Cadisch, G., 2011. Assessing the effect of organic residue quality on active decomposing fungi in a tropical Vertisol using ^{15}N-DNA stable isotope probing. Fungal Ecology 4, 115–119. doi:10.1016/j.funeco.2010.09.005

Fan, F., Yin, C., Tang, Y., Li, Z., Song, A., Wakelin, S.A., Zou, J., Liang, Y., 2014. Probing potential microbial coupling of carbon and nitrogen cycling during decomposition of maize residue by ^{13}C-DNA-SIP. Soil Biology and Biochemistry 70, 12–21. doi:10.1016/j.soilbio.2013.12.002

Fierer, N., Bradford, M.A.., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364. doi:10.1890/05-1839

Fuhrmann, I., He, Y., Lehndorff, E., Brüggemann, N., Amelung, W., Wassmann, R., Siemens, J., 2018. Nitrogen fertilizer fate after introducing maize and upland-rice into continuous paddy rice cropping systems. Agriculture, Ecosystems & Environment 258, 162–171. doi:10.1016/j.agee.2018.02.021

Furuhata, K., Kato, Y., Goto, K., Saitou, K., Sugiyama, J.-I., Hara, M., Fukuyama, M., 2008. Identification of Pink-Pigmented Bacteria Isolated from Environmental Water Samples and Their Biofilm Formation Abilities. Biocontrol Science 13, 33–39. doi:10.4265/bio.13.33

Gardes, G., Bruns, T., 1993. ITS primers with enhanced specificity for *Basidiomycetes* - application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118.

Gasoni, L., Stegman de Gurfinkel, B., 1997. The endophyte *Cladorrhinum foecundissimum*in cotton roots: phosphorus uptake and host growth. Mycological Research 101, 867–870. doi:DOI: undefined

Haichar, F.E.Z., Achouak, W., Christen, R., Heulin, T., Marol, C., Marais, M.F., Mougel, C., Ranjard, L., Balesdent, J., Berge, O., 2007. Identification of cellulolytic bacteria in soil by stable isotope probing. Environmental Microbiology 9, 625–634. doi:10.1111/j.1462-2920.2006.01182.x

He, Y., Siemens, J., Amelung, W., Goldbach, H., Wassmann, R., Alberto, M.C.R., Lücke, A., Lehndorff, E., 2015. Carbon release from rice roots under paddy rice and maize-paddy rice cropping. Agriculture, Ecosystems and Environment 210, 15–24. doi:10.1016/j.agee.2015.04.029

Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere: Biophysics, biogeochemistry and ecological relevance. Plant and Soil 321, 117–152. doi:10.1007/s11104-008-9885-9

Jilani, G., Akram, A., Ali, R.M., Hafeez, F.Y., Shamsi, I.H., Chaudhry, A.N., Chaudhry, A.G., 2007. Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. Annals of Microbiology 57, 177–184. doi:10.1007/BF03175204

Kjøller, A.H., Struwe, S., 2002. Fungal communities, succession, enzymes, and decomposition. Enzymes in the Environment: Activity, Ecology and Applications 1, 267–284.

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016. Resource partitioning between bacteria, fungi, and protists in the detritusphere of an agricultural soil. Frontiers in Microbiology 7, 1–12. doi:10.3389/fmicb.2016.01524

Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363–1371. doi:10.1016/j.soilbio.2010.04.003

Lee, C.G., Watanabe, T., Asakawa, S., 2017. Bacterial community incorporating carbon derived from plant residue in an anoxic non-rhizosphere soil estimated by DNA-SIP analysis. Journal of Soils and Sediments 17, 1084–1091. doi:10.1007/s11368-016-1621-0

Lee, C.G., Watanabe, T., Murase, J., Asakawa, S., Kimura, M., 2012. Growth of methanogens in an oxic soil microcosm: Elucidation by a DNA-SIP experiment using ^{13}C-labeled dried rice callus. Applied Soil Ecology 58, 37–44. doi:10.1016/j.apsoil.2012.03.002

Li, X., Shen, J., Yang, Z., Li, H., Wang, Y., 2017a. Control effect of different microbial agents in tobacco bacterial wilt. Plant Diseases and Pests 8, 35–38.

Li, X., Yang, C., Shen, J., 2017b. Effect of soil conditioner on the microbial community in the rhizosphere soil of tobacco. Agricultural Science & Technology 18, 834–838.

Lu, Y., Murase, J., Watanabe, A., Sugimoto, A., Kimura, M., 2004. Linking microbial community dynamics to rhizosphere carbon flow in a wetland rice soil. FEMS Microbiology Ecology 48, 179–186. doi:10.1016/j.femsec.2004.01.004

Lueders, T., Manefield, M., Friedrich, M.W., 2004. Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. Environmental Microbiology 6, 73–78. doi:10.1046/j.1462-2920.2003.00536.x

Lumini, E., Vallino, M., Alguacil, M.M., Romani, M., Bianciotto, V., 2011. Different farming and water regimes in Italian rice fields affect arbuscular mycorrhizal fungal soil communities. Ecological Applications : A Publication of the Ecological Society of America 21, 1696–1707.

Maarastawi, S.A., Frindte, K., Linnartz, M., Knief, C., 2018. Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of *Zea mays*. Frontiers in Microbiology. doi:10.3389/fmicb.2018.01295

Mau, R.L., Liu, C.M., Aziz, M., Schwartz, E., Dijkstra, P., Marks, J.C., Price, L.B., Keim, P., Hungate, B.A., 2015. Linking soil bacterial biodiversity and soil carbon stability. ISME Journal 9, 1477–1480. doi:10.1038/ismej.2014.205

McMurdie, P.J., Holmes, S., 2013. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8, 1–11. doi:10.1371/journal.pone.0061217

Murase, J., Matsui, Y., Katoh, M., Sugimoto, A., Kimura, M., 2006. Incorporation of ^{13}C-labeled rice-straw-derived carbon into microbial communities in submerged rice field soil and percolating water. Soil Biology and Biochemistry 38, 3483–3491. doi:10.1016/j.soilbio.2006.06.005

Murase, J., Shibata, M., Lee, C.G., Watanabe, T., Asakawa, S., Kimura, M., 2012. Incorporation of plant residue-derived carbon into the microeukaryotic community in a rice field soil revealed by DNA stable-isotope probing. FEMS Microbiology Ecology 79, 371–379. doi:10.1111/j.1574-6941.2011.01224.x

Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Aem 59, 695–700.

Nakamura, A., Tun, C.C., Asakawa, S., Kimura, M., 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipid fatty acid analysis. Biology and Fertility of Soils 38, 288–295. doi:10.1007/s00374-003-0658-6

Naz, I., Mirza, M., Bano, A., 2014. Molecular Characterization of Rhizosphere Bacterial Communities Associated With Wheat (Triticum Aestivum L.) Cultivars At. JAPS, Journal of Animal and Plant … 24, 1123–1134.

Neufeld, J.D., Vohra, J., Dumont, M.G., Lueders, T., Manefield, M., Friedrich, M.W., Murrell, J.C., 2007. DNA stable-isotope probing. Nature Protocols 2, 860–866. doi:10.1038/nprot.2007.109

Niu, X., Hao, X., Hong, Z., Chen, L., Yu, X., Zhu, X., 2015. A Putative Histone Deacetylase Modulates the Biosynthesis of Pestalotiollide B and Conidiation in Pestalotiopsis Microspora. Journal of Microbiology and Biotechnology 25, 579–588. doi:10.4014/jmb.1409.09067

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P.M., Stevens,

M.H.H., & Wagner, H., 2008. The vegan package. Community Ecology Package 190. doi:10.4135/9781412971874.n145

Panikov, N.S., 1995. Microbial Growth Kinetics. Chapman and Hall 1–67. doi:10.1016/B978-0-08-036131-4.50007-9

Pascault, N., Ranjard, Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Leveque, J., Mougel, C., Henault, C., Lemanceau, P., Pean, M., Boiry, S., Fontaine, S., Maron, P.A., 2013. Stimulation of Different Functional Groups of Bacteria by Various Plant Residues as a Driver of Soil Priming Effect. Ecosystems 16, 810–822. doi:10.1007/s10021-013-9650-7

Philippot, L., Raaijmakers, J.M., Lemanceau, P., Van Der Putten, W.H., 2013. Going back to the roots: The microbial ecology of the rhizosphere. Nature Reviews Microbiology 11, 789–799. doi:10.1038/nrmicro3109

Pietramellara, G., Franchi, M., Gallori, E., Nannipieri, P., 2001. Effect of molecular characteristics of DNA on its adsorption and binding on homoionic montmorillonite and kaolinite. Biology and Fertility of Soils 33, 402–409. doi:10.1007/s003740100341

Postma, J., Schilder, M.T., van Hoof, R.A., 2011. Indigenous Populations of Three Closely Related *Lysobacter spp.* in Agricultural Soils Using Real-Time PCR. Microbial Ecology 62, 948–958. doi:10.1007/s00248-011-9847-2

Qiang, X., Weiss, M., Kogel, K.H., Schäfer, P., 2012. *Piriformospora indica*-a mutualistic *basidiomycete* with an exceptionally large plant host range. Molecular Plant Pathology 13, 508–518. doi:10.1111/j.1364-3703.2011.00764.x

Qiu, Q., Conrad, R., Lu, Y., 2009. Cross-feeding of methane carbon among bacteria on rice roots revealed by DNA-stable isotope probing. Environmental Microbiology Reports 1, 355–361. doi:10.1111/j.1758-2229.2009.00045.x

Rui, J., Peng, J., Lu, Y., 2009. Succession of bacterial populations during plant residue decomposition in rice field soil. Applied and Environmental Microbiology 75, 4879–4886. doi:10.1128/AEM.00702-09

Sanguin, H., Remenant, B., Dechesne, A., Thioulouse, J., Vogel, T.M., Nesme, X., Moënne-Loccoz, Y., Grundmann, G.L., 2006. Potential of a 16S rRNA-based taxonomic microarray for analyzing the rhizosphere effects of maize on Agrobacterium spp. and bacterial communities. Applied and Environmental Microbiology 72, 4302–4312. doi:10.1128/AEM.02686-05

Shrestha, M., Shrestha, P.M., Conrad, R., 2011. Bacterial and archaeal communities involved in the in situ degradation of ^{13}C-labelled straw in the rice rhizosphere. Environmental Microbiology Reports 3, 587–596. doi:10.1111/j.1758-2229.2011.00267.x

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Roskot, N., Heuer, H., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis : plant-dependent enrichment and seasonal shifts revealed bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis. Applied and Environmental Microbiology 67, 4742–4751. doi:10.1128/AEM.67.10.4742

Soo, R.M., Woodcroft, B.J., Parks, D.H., Tyson, G.W., Hugenholtz, P., 2015. Back from the dead; the curious tale of the predatory cyanobacterium *Vampirovibrio chlorellavorus*. PeerJ 3, e968. doi:10.7717/peerj.968

Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R.S., Hess, W.M., Young, B., 1996. Taxol from *Pestalotiopsis microspora*, an endophytic fungus of Taxus wallachiana. Microbiology 142, 3–8. doi:10.1099/13500872-142-2-435

Tardy, V., Chabbi, A., Charrier, X., De Berranger, C., Reignier, T., Dequiedt, S., Faivre-Primot, C., Terrat, S., Ranjard, L., Maron, P.A., 2015. Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. PLoS ONE 10, 1–17. doi:10.1371/journal.pone.0130672

Töwe, S., Wallisch, S., Bannert, A., Fischer, D., Hai, B., Haesler, F., Kleineidam, K., Schloter, M., 2011. Improved protocol for the simultaneous extraction and column-based separation of DNA and RNA from different soils. Journal of Microbiological Methods 84, 406–412. doi:10.1016/j.mimet.2010.12.028

Tuong, T.P., Bouman, B. a. M., Mortimer, M., 2005. More rice, less water-integrated approaches for increasing water productivity in irrigated rice-based systems in asia. Plant Production Science 8, 231–241. doi:10.1626/pps.8.231

Uhlík, O., Jecná, K., Leigh, M.B., Macková, M., Macek, T., 2009. DNA-based stable isotope

probing: a link between community structure and function. Science of the Total Environment 407, 3611–3619. doi:10.1016/j.scitotenv.2008.05.012

Wang, Y., Lai, Z., Li, X.X., Yan, R.M., Zhang, Z. Bin, Yang, H.L., Zhu, D., 2016. Isolation, diversity and acetylcholinesterase inhibitory activity of the culturable endophytic fungi harboured in *Huperzia serrata*from Jinggang Mountain, China. World Journal of Microbiology and Biotechnology 32. doi:10.1007/s11274-015-1966-3

Watanabe, A., Katoh, K., Kimura, M., 1993. Effect of rice straw application on CH_4 emission from paddy fields. Soil Science and Plant Nutrition 39, 701–706. doi:10.1080/00380768.1993.10419187

Watanabe, A., Yoshida, M., Kimura, M., 1998. Contribution of rice straw carbon to CH_4emission from rice paddies using ^{13}C-enriched rice straw 103, 8237–8242.

Weller, S., Kraus, D., Ayag, K.R.P., Wassmann, R., Alberto, M.C.R., Butterbach-Bahl, K., Kiese, R., 2015. Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. Nutrient Cycling in Agroecosystems 101, 37–53. doi:10.1007/s10705-014-9658-1

White, T.J., Bruns, S., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications. doi:citeulike-article-id:671166

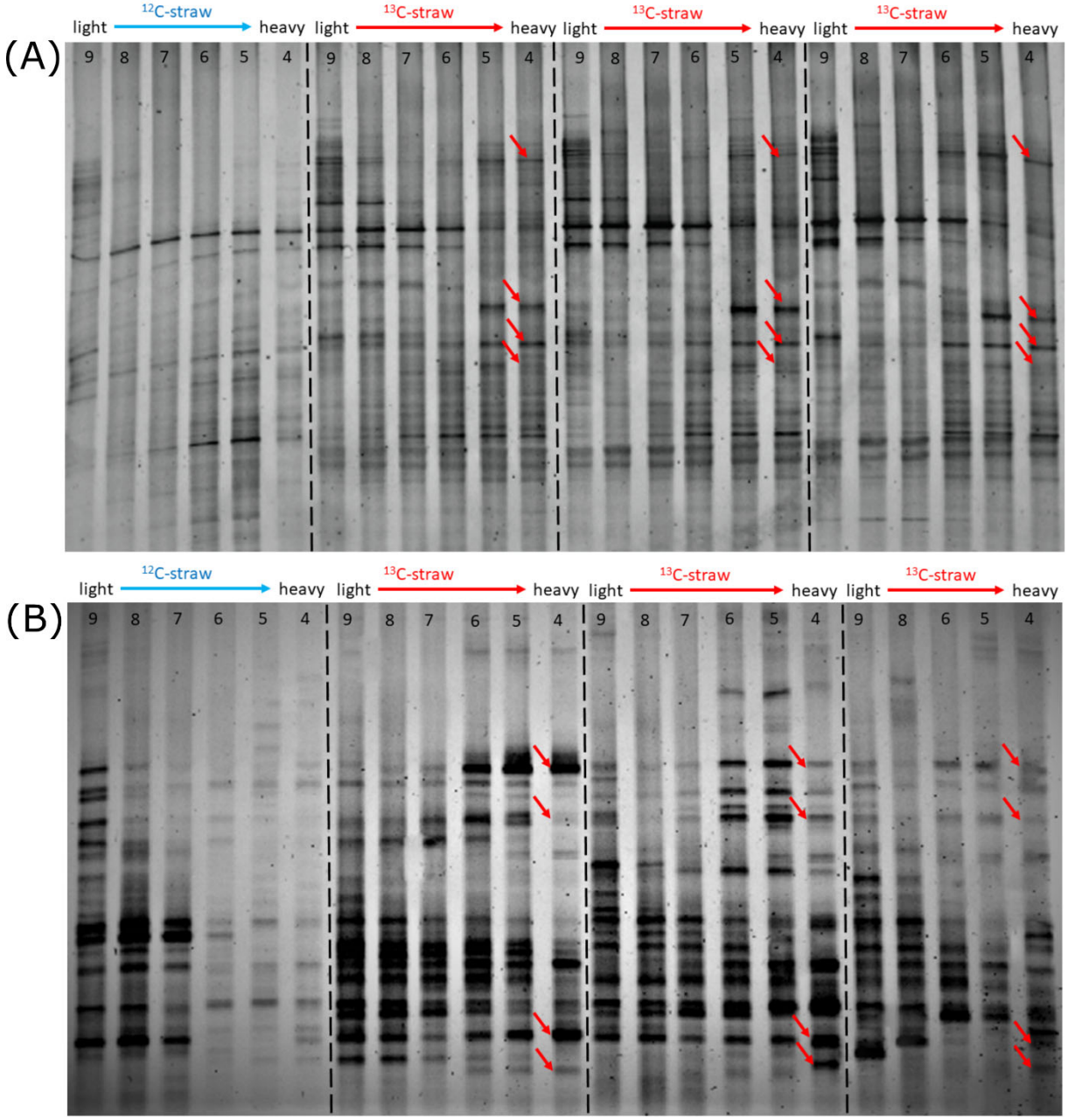
Yang, C., Crowley, D.E., 2000. Rhizosphere Microbial Community Structure in Relation to Root Location and Plant Iron Nutritional Status Rhizosphere Microbial Community Structure in Relation to Root Location and Plant Iron Nutritional Status 66, 345–351. doi:10.1128/AEM.66.1.345-351.2000.Updated

Zhang, H., Zhou, S., 2016. Screening and cultivation of oligotrophic aerobic denitrifying bacteria. The Handbook of Environmental Chemistry 38, 1–514. doi:10.1007/978-3-319-20391-1

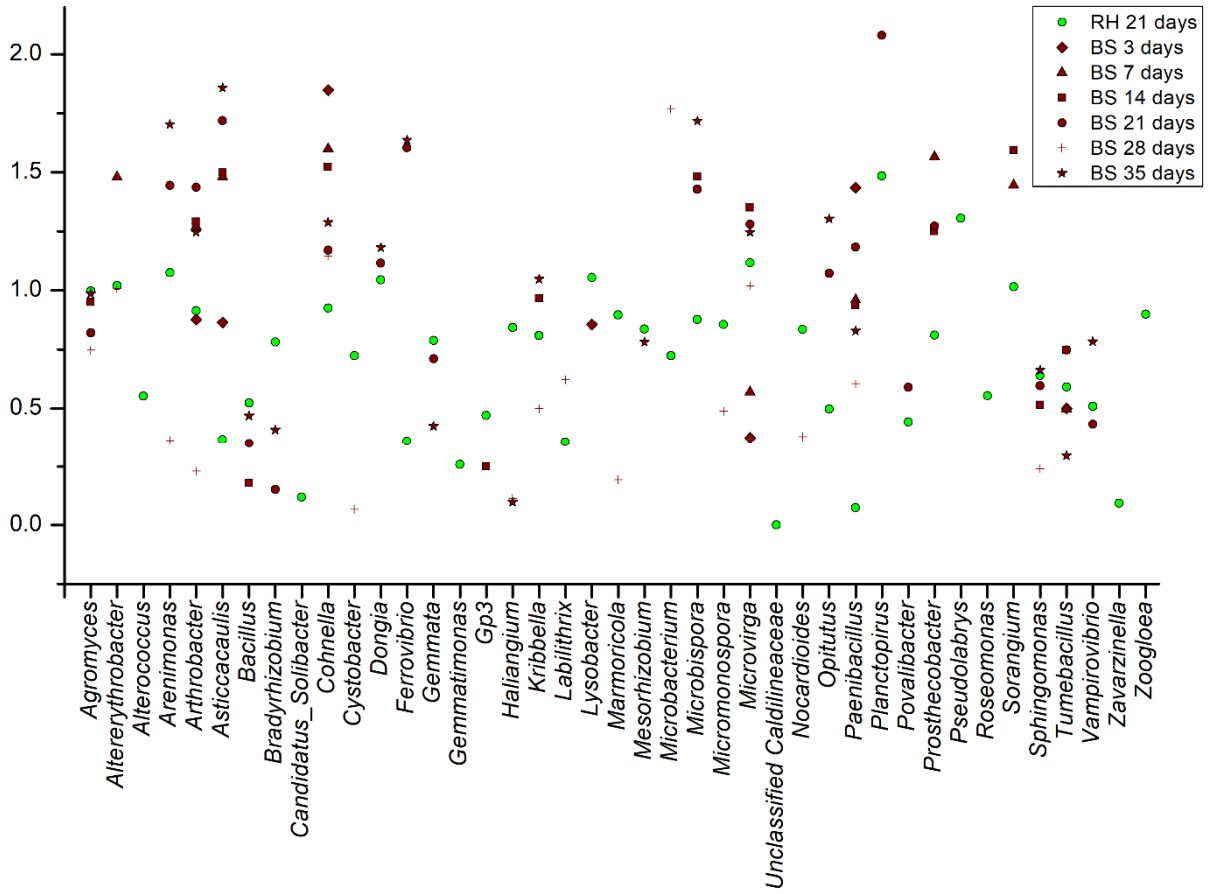
Zhao, M., Sun, B., Wu, L., Gao, Q., Wang, F., Wen, C., Wang, M., Liang, Y., Hale, L., Zhou, J., Yang, Y., 2016. Zonal soil type determines soil microbial responses to maize cropping and fertilization. MSystems 1, e00075-16. doi:10.1128/mSystems.00075-16

Supplementary figures and tables

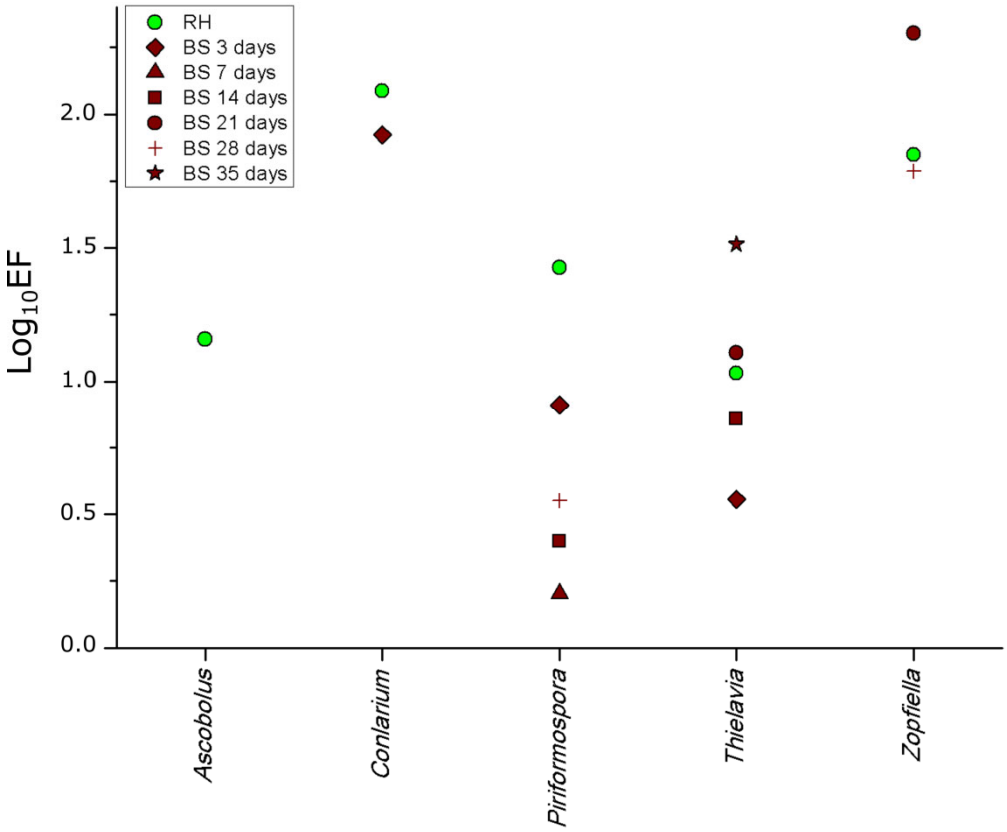
Supplementary figures



Supplementary figure 1: DGGE fingerprints of the 16S rRNA gene (A) and ITS1 region (B) PCR products amplified from density fractionated DNA of the rhizosphere samples after 21 days of incubation with unlabelled or ^{13}C-labelled straw. Gradient fractions with increasing buoyant density from light (fraction 9) to heavy (fraction 4) were loaded for one pooled ^{12}C-sample and the three individual replicate ^{13}C-samples. The gels show differences between I.) ^{12}C-heavy and ^{13}C-heavy fractions and II.) ^{13}C-light and ^{13}C-heavy fractions and thus label incorporation. Red arrows highlight bands with higher intensity in the ^{13}C-heavy fractions.



Supplementary figure 2: ^{13}C-labelled bacterial genera identified in rhizosphere and bulk soil samples after different incubation periods. Bulk soil samples are displayed with differently shaped brown symbols according to the days of incubation and rhizosphere sample as green dots. EFs were $\log_{10}$ transformed and are shown on the y-axis. To reduce complexity, 62 genera that were only detected in bulk soil samples but not in the rhizosphere were excluded from the diagram.



Supplementary figure 3: ^{13}C-labelled fungal genera identified in rhizosphere and bulk soil samples after different incubation periods. Bulk soil samples are displayed with differently shaped brown symbols according to the days of incubation and rhizosphere sample as green dots. EFs were $\log_{10}$ transformed and are shown on the y-axis. To reduce complexity, 12 genera that were only detected in bulk soil samples but not in the rhizosphere were excluded from the diagram.

Supplementary tables

Supplementary Table 1: Relative abundance (%) of bacterial genera in the heavy fraction of ^{13}C-labelled samples and EF of labelled genera, identified in bulk soil samples after 3, 7, 14, 21, 28 and 35 days of incubation and in the rhizosphere sample after 21 days. EF = enrichment factor, RA = relative abundance (%).

	Bulk soil 3 d		Bulk soil 7 d		Bulk soil 14 d		Bulk soil 21 d		Bulk soil 28 d		Bulk soil 35 d		Rhizopshere 21 d	
Genus	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**
Aeromonas	22.8	2.19	9.1	0.16					36.5	0.21				
Agromyces					8.9	0.69	6.6	0.68	5.5	0.44	9.6	0.41	9.9	0.78
Alsobacter					11.8	0.16	2.9	0.16	5.7	0.10	3.0	0.14		
Altererythrobacter			30.0	0.11					10.1	0.11			10.4	0.11
Alterococcus													3.5	0.12
Aquaspirillum			29.4	0.16										
Aquicella									1.1	0.17	1.8	0.16		
Arenimonas							27.8	0.50	2.3	0.25	50.4	0.37	11.8	0.71
Unclassified Armatimonadetes_gp4					3.0	0.16								
Arthrobacter	7.5	0.76	18.6	0.83	19.5	0.59	27.3	0.92	1.7	0.35	17.6	0.51	8.1	0.58
Asticcacaulis	7.3	0.19	30.2	0.47	31.4	0.51	52.2	1.09			72.0	0.59	2.3	0.56
Azoarcus			33.3	0.36			5.3	0.23	34.2	0.74	8.7	0.15		
Azospira			3.1											
Azospirillum							2.2	0.16						
Bacillus					1.5	17.48	2.2	12.65			2.9	21.33	3.3	7.93
Bdellovibrio	3.2	0.16	5.5	0.23										
Bradyrhizobium							1.4	0.11			2.5	0.21	6.0	0.23
Brevundimonas	24.4	0.52	69.0	0.16	64.0	0.13								
Burkholderia									14.0	0.10	2.2	0.11		
Candidatus_Solibacter													1.3	0.12
Caulobacter	36.1	3.88	25.5	1.86	36.9	0.89	20.4	0.85			14.1	0.35		
Cellvibrio	2.8	16.34	43.6	11.94					13.8	1.22				

Genus	Bulk soil 3 d		Bulk soil 7 d		Bulk soil 14 d		Bulk soil 21 d		Bulk soil 28 d		Bulk soil 35 d		Rhizopshere 21 d	
	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)
Chitinophaga			32.6	2.05	19.6	1.45	4.6	0.52	1.7	0.23				
Cohnella	70.3	6.31	39.5	3.32	33.3	1.39	14.7	1.21	13.9	2.21	19.3	0.48	8.4	0.13
Conexibacter														
Cupriavidus											33.48	0.10		
Cystobacter									1.1	0.43			5.2	0.34
Cytophaga							10.4	0.40	4.9	0.46				
Desulfitobacterium									1.2	0.18				
Desulfosporosinus									8.1	0.24				
Devosia	38.0	1.14	41.3	1.02	27.4	0.54	16.4	0.45			38.2	0.73		
Dongia							13.0	0.31			15.1	0.21	11.0	0.23
Dyadobacter			24.2	0.17	21.3	0.10								
Dyella							6.3	0.25	22.7	0.44	21.7	0.40		
Exiguobacterium			16.0	0.12										
Ferrovibrio							40.0	0.14			43.2	0.31	2.3	0.21
Flavobacterium									1.3	0.20				
Fluviicola					5.7	0.16			1.2	0.15				
Gaiella														
Gemmata							5.1	0.31			2.6	0.16	6.1	0.26
Gemmatimonas													1.8	0.77
Unclassified *Acidobacteria_Gp16*									1.4	0.49				
Unclassified *Acidobacteria_Gp3*					1.7	0.67							2.9	0.61
Haliangium									1.3	0.45	1.2	0.32	6.9	1.07
Hamadaea					4.8	0.10								
Kribbella					9.2	0.17			3.1	0.13	11.1	0.50	6.4	0.19
Ktedonobacter											4.7	0.11		

Genus	Bulk soil 3 d		Bulk soil 7 d		Bulk soil 14 d		Bulk soil 21 d		Bulk soil 28 d		Bulk soil 35 d		Rhizopshere 21 d	
	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)
Labilithrix									4.1	0.16			2.2	0.13
Legionella							3.1	0.15						
Lysobacter	7.1	0.20											11.3	0.13
Marmoricola									1.5	0.20			7.8	0.45
Massilia	1.9	1.72	6.4	1.03	16.8	0.63	5.2	0.37	1.0	0.23				
Melioribacter	1.92	0.19												
Mesorhizobium											6.0	0.10	6.8	0.13
Methylophilus			28.1	0.22										
Microbacterium									58.7	0.12			5.2	0.11
Microbispora					30.3	1.94	26.8	1.77			52.1	0.95	7.5	0.96
Micromonospora									3.08	0.33			7.16	0.64
Microvirga	2.37	0.97	3.7	1.38	22.0	1.73	19.1	2.19	10.4	2.29	17.5	1.38	13.0	2.07
Mucilaginibacter			11.0	0.63			7.2	0.48	10.4	0.74	12.6	0.52		
Mycobacterium											1.9	0.13		
Unclassified *Caldilineaceae*													1.0	1.81
Niastella							3.5	0.22						
Nocardioides									2.3	0.14			6.8	0.28
Nonomuraea											36.3	0.15		
Novosphingobium	7.2	1.11	10.4	0.62	10.9	0.22	3.1	0.14						
Ochrobactrum			146	0.15										
Ohtaekwangia											41.8	0.44		
Opitutus							11.7	1.47			20.0	0.84	3.1	2.29
Oxalophagus					1.6	0.44	2.1	0.37			2.2	0.51		
Paenibacillus	27.1	10.76	9.09	2.92	8.6	4.83	15.2	8.73	4.0	10.97	6.7	3.19	1.1	1.49
Paucimonas	1.6	0.17												
Pedobacter			14.9	0.11										
Phenylobacterium					3.3	0.64			2.2	0.51	8.6	0.71		

Genus	Bulk soil 3 d		Bulk soil 7 d		Bulk soil 14 d		Bulk soil 21 d		Bulk soil 28 d		Bulk soil 35 d		Rhizopshere 21 d	
	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)
Planctopirus							119	0.45					30.4	0.10
Pleomorphomonas	11.3	0.10	34.7	0.19										
Porphyrobacter							9.5	0.17	4.2	0.18	19.4	0.30		
Povalibacter							3.8	0.33					2.7	0.17
Prosthecobacter			36.6	0.46	17.7	0.19	18.7	0.26					6.4	0.14
Pseudoduganella	32.6	3.98	21.9	1.95	17.7	0.82	8.1	0.34						
Pseudogulbenkiania			61.8	0.16										
Pseudolabrys													20.1	0.15
Pseudonocardia					18.6	0.12								
Pseudoxanthomonas	16.0	0.35	38.5	0.32	27.9	0.28	62.7	0.11			150	0.81		
Ralstonia	1.0	0.16	4.8	0.24	6.4	0.21	8.1	0.32	2.3	0.20				
Ramlibacter					5.8	1.30	1.7	1.64	1.0	0.62				
Reyranella											8.0	0.24		
Rhizobium	27.2	2.77	49.8	3.72	28.8	1.49	21.9	1.35			18.1	0.52		
Roseomonas													3.5	0.19
Rubrobacter														
Saccharibacteria_ genera_inc_sedis					3.17	1.58	4.31	3.04						
Simplicispira	19.5	0.21	61.1	0.31										
Singulisphaera					1.2	0.13					11.3	0.32		
Sinomonas					36.0	0.16			55.7	0.13	30.6	0.23		
Solirubrobacter														
Sorangium			27.9	1.76	39.2	0.34							10.3	0.11
Sphingobium	14.4	0.11	22.1	0.21	26.5	0.11								
Sphingomonas					3.2	1.16	3.9	1.75	1.7	1.09	4.5	1.30	4.3	2.04
Sphingopyxis					32.5	0.16			17.9	0.10				
Spirochaeta							6.0	0.10						

Genus	Bulk soil 3 d		Bulk soil 7 d		Bulk soil 14 d		Bulk soil 21 d		Bulk soil 28 d		Bulk soil 35 d		Rhizopshere 21 d	
	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)	EF	RA (%)
Stenotrophomonas	19.2	1.87	6.8	0.72	26.6	0.26	19.4	0.23						
Streptomyces									2.8	0.55	6.8	3.53		
Tumebacillus	3.1	0.10	3.1	0.12	5.5	0.11	5.60	0.48			1.9	0.14	3.9	0.41
Vampirovibrio							2.7	0.21			6.0	0.23	3.2	0.95
Zavarzinella													1.2	0.10
Zoogloea													7.9	0.14

Supplementary Table 2: Relative abundance (%) of fungal genera in the heavy fraction of ^{13}C-labelled samples and EF of labelled genera, identified in bulk soil samples after 3, 7, 14, 21, 28 and 35 days of incubation and in the rhizosphere sample after 21 days. EF = enrichment factor, RA = relative abundance (%).

	Bulk soil 3 d		**Bulk soil 7 d**		**Bulk soil 14 d**		**Bulk soil 21 d**		**Bulk soil 28 d**		**Bulk soil 35 d**		**Rhizopshere 21 d**	
Genus	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**	**EF**	**RA (%)**
Ascobolus													14.3	0.21
Chaetospermum	2.0	0.22												
Cladorrhinum							27.8	0.77	8.7	0.76	5.9	0.24		
Clitopilus											1.0	0.16		
Conlarium	83.6	0.19											122	0.25
Curvularia											4.2	0.55		
Fusarium	3.7	15.29												
Malassezia	38.1	0.16												
Microdochium	1.3	0.37												
Myrmecridium	2.5	0.17												
Myrothecium					13.8	1.16			38.7	2.63				
Periconia											10.1	0.71		
Pestalotiopsis									13.9	0.24				
Piriformospora	8.1	2.39	1.5	2.50	2.5	0.81			3.5	0.92			26.6	1.09
Rhizophlyctis	13.1	1.28					2.6	4.53						
Thielavia	3.5	2.15			7.2	3.84	12.8	2.69			32.6	7.32	10.7	0.57
Zopfiella							200	0.40	61.2	0.21			70.6	0.20

2.4. Rice straw serves as additional carbon source for rhizosphere microorganisms (manuscript 4)

Sarah A. Maarastawi[1], Katharina Frindte [1], Paul L. E. Bodelier[2], Claudia Knief [1,*]

[1] Institute for Crop Science and Resource Conservation (INRES), Molecular Biology of the Rhizosphere, University of Bonn, Bonn, Germany

[2] Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands.

Author contributions:

SM and CK conceived the research. SM designed the research. SM performed the experiments. SM analysed the data with help of CK, PB and KF. SM wrote the paper. SM, KF and CK approved the paper.

Planned submission to Soil biology and biochemistry

Abstract

Straw application is a common agricultural fertilizer practice, providing an additional carbon and nutrient source for soil microorganisms. We investigated the influence of rice straw application on root exudate consuming microorganisms in the rhizosphere of *Zea mays* based on $^{13}CO_2$ pulse labelling and phospholipid fatty acid stable isotope probing (PLFA-SIP). The application of straw resulted in approx. 51 % decrease in labelling of PLFAs in the rhizosphere of 30 and 40 day old plants. This decrease was partially explained by a lower rate of CO_2 assimilation by the plant in the presence of rice straw. Additionally, the uptake of root exudates by rhizosphere organisms was decreased in the presence of rice straw, which can obviously serve as additional carbon source for rhizosphere microorganisms.

The rhizosphere is defined as the thin soil layer surrounding plant roots (Baudoin et al., 2003; Peiffer et al., 2013). Due to the release of rhizodeposits a physically, chemically and biologically different environment develops in the rhizosphere in comparison to bulk soil, along with different functional abilities of the microbial communities residing in these two compartments (Baudoin et al., 2003; Bowen and Rovira, 1999). Rhizodeposition encompasses the release of exudates as well as of dead cell material by plant roots (Baudoin et al., 2001; Lynch and Whipps, 1990). This leads to a higher carbon concentration in the rhizosphere than in bulk soil, which supports a higher microbial activity (Baudoin et al., 2002; Bowen and Rovira, 1999). The composition of rhizodeposits varies in quantity and quality in depending on plant species or variety, developmental stage and plant growth conditions (Badri and Vivanco, 2009; Schilling et al., 1998; Swinnen et al., 1994; van Veen et al., 1991). This variation leads to the development of specific microbial communities (Badri and Vivanco, 2009; Berg and Smalla, 2009; Bulgarelli et al., 2012).

Straw application is discussed as effective management practice in rice paddies under crop rotation to return nutrients and reduce crack formation (Asari et al., 2007; Cabangon and Tuong, 2000). The addition of straw leads to changes in the microbial community, not only in the bulk soil, but also in the rhizosphere (Maarastawi et al., 2018), suggesting that rhizosphere microorganisms benefit from rhizodeposits as well as from straw-derived carbon. However, it is unclear to what extent the application of rice straw may influence the uptake of root exudates by microorganisms. To address this question and to identify root exudate metabolizing microbial groups that respond to straw application, we performed $^{13}CO_2$ stable isotope labelling of maize plants, cultivated in paddy fields under rice-maize crop rotation with and without rice straw, respectively. Label incorporation in the microbial community was analysed using phospholipid fatty acids (PLFA) as marker.

The study site was located at the International Rice Research Institute in Los Baños, Philippines (14°11'N, 121°15'E). Two fields managed under maize-rice crop rotation since 4 years were chosen. The straw was applied in two steps to prevent crack formation efficiently: 300 $g*m^{-3}$ of dried and chopped rice straw was incorporated into the soil, and 300 $g*m^{-3}$ of straw were applied as mulch on the soil surface six weeks later. Maize was planted one day after the second straw application in the dry season (at the beginning of December 2015). The maize plants were watered every day and received basal fertilization according to Maarastawi et al. (2018).

The $^{13}CO_2$ labelling of maize plants was conducted in triplicates for each treatment (i. e. with and without straw). Cylindrical chambers (50 cm∅) consisting of an aluminium frame covered with a transparent polyethylene foil (thickness: 180 µm; light transmission: 90%), were used to

cover one plant each. The volume of the chambers was 100 or 200 dm^3, depending on the growth stage of the plant. A fan was positioned inside the chamber to ensure air circulation. The release of $^{13}CO_2$ from 99 % ^{13}C-enriched 0.3 M Na_2CO_3 solution in the chambers was induced by addition of 2 M H_2SO_4. Several pulses were applied per day, depending on the growth stage of the plant, to reach a CO_2 concentration of approximately 400 ppmv. Plants were labelled 15, 25 or 35 days after seeding on three consecutive days for six hours per day. Rhizosphere samples were collected 2.5 days after the last labelling event (i. e. 20, 30 and 40 days after seeding).

Rhizosphere samples were collected according to Maarastawi et al., (2018). PLFA extraction was done according to Knief *et al.* (2003). Fatty acid methyl esters (FAMEs) were separated by gas chromatography, identified and quantified using a flame ionization detector and an isotope ratio mass spectrometer (IRMS) was used for the quantification of ^{13}C-label incorporation according to (Morriën et al., 2017). For each PLFA, ^{13}C-label incorporation was calculated according to Knief et al. (2003). Thereby, the $\delta^{13}C$ values of the FAMEs were corrected for the addition of the extra carbon atom introduced during methylation (-33.46 ± 0.02). The complete biomass of the labelled and unlabelled plants was dried and ground to a fine powder. The $\delta^{13}C$ [‰] isotopic signature was determined with an elemental analyser coupled with a Delta V Advantage isotope ratio mass spectrometer according to He et al., (2015). To quantify the assimilation rate and incorporation of ^{13}C into the plant biomass, we calculated the ^{13}C excess according to Epron et al. (2011).

Label incorporation into the plant biomass was influenced by the plant developmental stage. Plants with an age of 30 days incorporated significantly more ^{13}C into their biomass than 20 and 40 day old plants (two-way ANOVA with Tukey post-hoc tests, $P < 0.001$; Figure 1A). Previous studies with maize conducted without straw addition confirm these results, showing the highest growth rate of maize in the same developmental stage (Bu et al., 2013; Plénet et al., 2000). Straw application had a low impact on label incorporation into the maize plants, a significant decrease in labelling was only observed after 30 days (one-way ANOVA, $P < 0.01$) (Figure 1A). This indicates that the applied straw reduces the metabolic activity of the plant specifically at this growth stage. Straw application will stimulate the growth of microorganisms (Fontaine et al., 2003; Kuzyakov, 2010), which may lead to a stronger competition for nutrients between plants and microorganisms in the rhizosphere, becoming a disadvantage for the plant at this stage of high biomass production (Kuzyakov and Xu, 2013).

The detected ^{13}C-excess in the plants correlated well with the amount of label incorporated into the PLFAs of the rhizosphere microbiota (Figure 1B). The highest labelling of root exudate

consuming microbes was detected after 30 days. At this time point, the label incorporation into the rhizosphere microbiota made up about 1/15 - 1/20 of the plant label (Figure 1C), suggesting that plants had the highest root exudation rates at this stage or released a blend of carbon compounds that could be more rapidly metabolized by the rhizosphere microbiota than that released at the other time points (Baudoin et al., 2003; Butler et al., 2003). In the presence of straw, the incorporation and ratio of ^{13}C-label into the rhizosphere microbiota was significantly lower (two-way ANOVA, $P < 0.001$; Figure 1B, C) at all time points. This indicates that the addition of straw stimulates the rhizosphere microbiota to use carbon derived from straw as additional source and simultaneously reduces the uptake of root exudates.

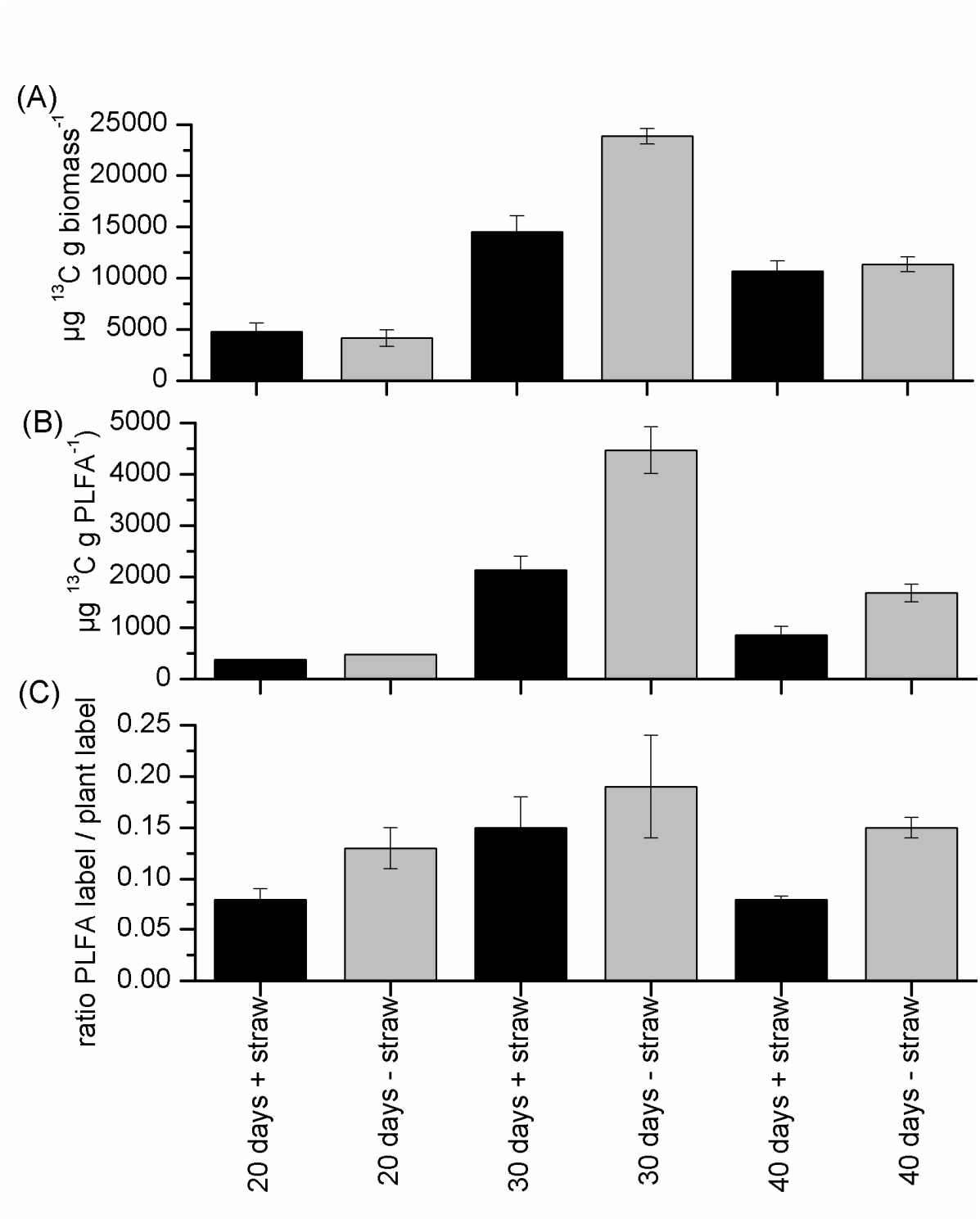


Figure 1: Label intensity of plant biomass (A) and of PLFAs of rhizoshere microorganisms (B). Label intensity is presented as mean of three samples ± standard error, with exception of the samples taken at day 20. These were pooled to get adequate amounts of material for PLFA extraction. Ratio of ^{13}C-label intensity between microbial PLFAs and plant biomass, were calculated from the values of ^{13}C-excess in plants (µg ^{13}C g biomass^{-1}) and of ^{13}C-label in rhizosphere microorganisms (µg ^{13}C g PLFA^{-1}) (C) ± standard error.

Analysis of the individual PLFAs (Figure 2) revealed that only a few PLFAs showed a lower labelling in the presence of straw in the rhizosphere of 20-day old plants. Especially 16:0 and 18:1ω9c, which showed the strongest label reduction in dependence on straw, are quite common PLFAs in diverse microorganisms (Lange et al., 2014; Willers et al., 2015). Several other PLFAs showed a slightly higher labelling in the presence of straw, including 18:0, and 18:2ω6c, which suggests that the corresponding microorganisms profited more efficiently from root exudates. The shift of some root exudate consuming microbes to carbon compounds derived from straw may have led to a lowered competition pressure for some other root exudate consuming microbes and therefore to a higher label incorporation.

At the later time points, all PLFAs showed a reduced labelling in the presence of straw, indicating that diverse microorganisms in the rhizosphere were affected by the straw addition, leading to a reduced use of root exudates. At these later time points, a broad range of carbon compounds from straw might have been available. Straw degradation is known to follow a temporal succession with a rapid degradation of labile substances within the initial phase, followed by a slower degradation of more complex compounds by oligotrophic organisms, which again release breakdown products that support the growth of further microorganisms (Bastian et al., 2009; Bernard et al., 2007). Thus, microorganisms in the rhizosphere may have profited from the more complex carbon compounds provided by the straw incorporated into the soil, as well as from degradation products, at the later time points.

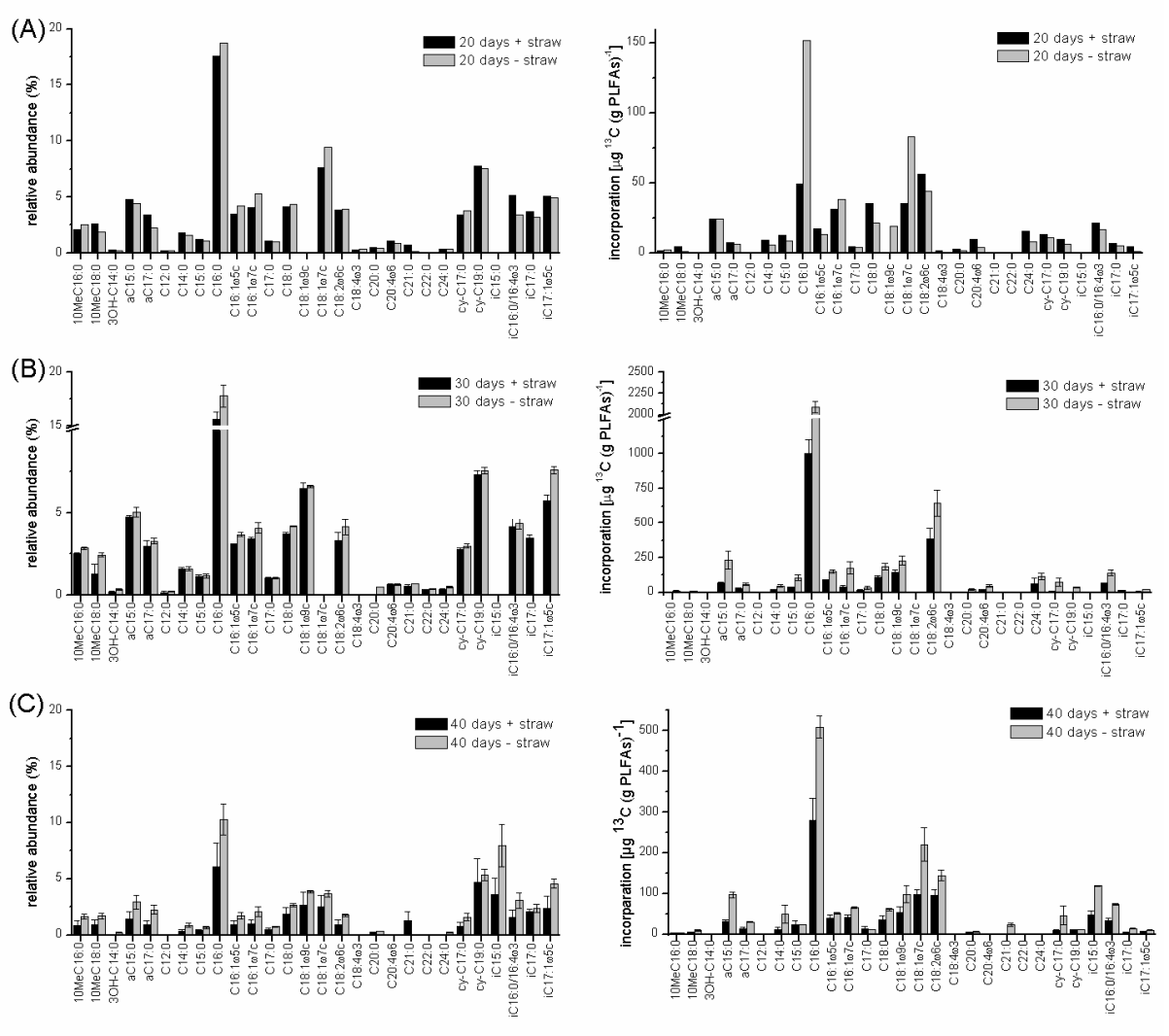


Figure 2: PLFA profiles of the rhizosphere microbiota displaying the relative abundance of all PLFAs (left panel, community profile) compared to the ^{13}C-labelled PLFAs (right panel; activity profile) after (A) 20 days, (B) 30 days and (C) 40 days. The incorporation of ^{13}C in µg per g PFLA was calculated with the relative abundance and δ^{13} C value. Data of relative abundance and label incorporation are means of three samples ± standard error, with exception of the 20 day old samples. 20 day old rhizosphere soil samples were pooled to get adequate amounts of material for the PLFA extraction.

The PLFA 16:1ω5c, which is characteristic for arbuscular fungi (Willers et al., 2015), showed a labelling pattern that reflects very closely the labelling of the plant biomass (Figure 1). Those fungi are known to be strongly influenced by plant derived carbon (Bais et al., 2006), which explains the comparable labelling pattern. However, mycorrhizal fungi are also known as oligotrophs being able to decompose complex carbon compounds in soil (Hodge et al., 2001). The strong reduction in labelling at day 30 indicates that they were affected by the straw application, possibly profiting to some extent from the straw derived carbon (Bais et al., 2006; Nakamura et al., 2003).

Similarly, the PLFAs 16:0, 18:2ω6c, and a15:0 showed the strongest decrease in labelling in dependence on straw at day 30 (Figure 2), when plant carbon assimilation was most strongly affected by straw application (Figure 1). The PLFA a15:0 is indicative for Gram-positive bacteria (Willers et al., 2015), while 18:2ω6, 9c, is an indicator for saprotrophic fungi (Hannula et al., 2012), often discussed as marker in combination with 18:1ω9c (Balasooriya et al., 2014). The reduced labelling of these PLFAs suggests that these bacteria and fungi have probably shifted to some extent from root exudate to rice straw carbon, as they may possibly be less competitive compared to bacteria in taking up root exudates.

In summary, our study shows that the temporal dynamic of CO_2 assimilation by plants is reflected by the rhizosphere microbiota assimilating root exudates. However, decrease in microbial PLFA labelling cannot fully be explained by reduced plant carbon assimilation (Figure 1C). An additional reduction in labelling is resulting from the use of straw as additional carbon source. The uptake of root exudates is thus influenced by additional carbon sources in the rhizosphere.

References

Asari, N., Ishihara, R., Nakajima, Y., Kimura, M., Asakawa, S., 2007. Succession and phylogenetic composition of eubacterial communities in rice straw during decomposition on the surface of paddy field soil. Soil Science and Plant Nutrition 53, 56–65. doi:10.1111/j.1747-0765.2007.00110.x

Badri, D. V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant, Cell and Environment 32, 666–681. doi:10.1111/j.1365-3040.2009.01926.x

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57, 233–266. doi:10.1146/annurev.arplant.57.032905.105159

Balasooriya, W.K., Denef, K., Huygens, D., Boeckx, P., 2014. Translocation and turnover of rhizodeposit carbon within soil microbial communities of an extensive grassland ecosystem. Plant and Soil 376, 61–73. doi:10.1007/s11104-012-1343-z

Bastian, F., Bouziri, L., Nicolardot, B., Ranjard, L., 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biology and Biochemistry 41, 262–275. doi:10.1016/j.soilbio.2008.10.024

Baudoin, E., Benizri, E., Guckert, A., 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology and Biochemistry 35, 1183–1192. doi:10.1016/S0038-0717(03)00179-2

Baudoin, E., Benizri, E., Guckert, A., 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Applied Soil Ecology 19, 135–145. doi:10.1016/S0929-1393(01)00185-8

Baudoin, E., Benizri, E., Guckert, A., 2001. Metabolic fingerprint of microbial communities from distinct maize rhizosphere compartments. European Journal of Soil Biology 37, 85–93. doi:10.1016/S1164-5563(01)01071-8

Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68, 1–13. doi:10.1111/j.1574-6941.2009.00654.x

Bernard, L., Mougel, C., Maron, P.A., Nowak, V., Lévêque, J., Henault, C., Haichar, F.E.Z., Berge, O., Marol, C., Balesdent, J., Gibiat, F., Lemanceau, P., Ranjard, L., 2007. Dynamics and identification of soil microbial populations actively assimilating carbon from 13C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. Environmental Microbiology 9, 752–764. doi:10.1111/j.1462-2920.2006.01197.x

Bowen, G.D., Rovira, A.D., 1999. The Rhizosphere and its management to improve plant growth, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–102. doi:https://doi.org/10.1016/S0065-2113(08)60425-3

Bu, L.D., Liu, J.L., Zhu, L., Luo, S.S., Chen, X.P., Li, S.Q., Lee Hill, R., Zhao, Y., 2013. The effects of mulching on maize growth, yield and water use in a semi-arid region. Agricultural Water Management 123, 71–78. doi:10.1016/j.agwat.2013.03.015

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F.O., Amann, R., Eickhorst, T., Schulze-Lefert, P., 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488, 91–95. doi:10.1038/nature11336

Butler, J.L., Williams, M. a, Bottomley, P.J., Myrold, D.D., 2003. Microbial community dynamics associated with rhizosphere carbon flow microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793–6800. doi:10.1128/AEM.69.11.6793

Cabangon, R.J., Tuong, T.P., 2000. Management of cracked soils for water saving during land preparation for rice cultivation. Soil and Tillage Research 56, 105–116. doi:10.1016/S0167-1987(00)00125-2

Dunfield, P.F., Knief, C., Lipski, A., 2003. Diversity and Activity of Methanotrophic Bacteria. American Society for Microbiology 69, 6703–6714. doi:10.1128/AEM.69.11.6703

Epron, D., Ngao, J., Dannoura, M., Bakker, M.R., Zeller, B., Bazot, S., Bosc, A., Plain, C., Lata, J.C., Priault, P., Barthes, L., Loustau, D., 2011. Seasonal variations of belowground carbon transfer assessed by in situ 13CO2 pulse labelling of trees. Biogeosciences 8, 1153–1168. doi:10.5194/bg-8-1153-2011

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: A question of microbial competition? Soil Biology and Biochemistry 35, 837–843. doi:10.1016/S0038-0717(03)00123-8

Hannula, S.E., Boschker, H.T.S., de Boer, W., van Veen, J.A., 2012. 13C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a genetically starch-modified potato (Solanum tuberosum) cultivar and its parental isoline. New Phytologist 194, 784–799. doi:10.1111/j.1469-8137.2012.04089.x

He, Y., Siemens, J., Amelung, W., Goldbach, H., Wassmann, R., Alberto, M.C.R., Lücke, A., Lehndorff, E., 2015. Carbon release from rice roots under paddy rice and maize-paddy rice cropping. Agriculture, Ecosystems and Environment 210, 15–24. doi:10.1016/j.agee.2015.04.029

Henneberger, R., Chiri, E., Bodelier, P.E.L., Frenzel, P., Lüke, C., Schroth, M.H., 2015. Field-scale tracking of active methane-oxidizing communities in a landfill cover soil reveals spatial and seasonal variability. Environmental Microbiology 17, 1721–1737. doi:10.1111/1462-2920.12617

Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material 297–299.

Knief, C., Altendorf, K., Lipski, A., 2003. Linking autotrophic activity in environmental samples with specific bacterial taxa by detection of 13C-labelled fatty acids. Environmental Microbiology 5, 1155–1167. doi:10.1046/j.1462-2920.2003.00510.x

Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry 42, 1363–1371. doi:10.1016/j.soilbio.2010.04.003

Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen : mechanisms and ecological relevance. New Phytologist 198, 656–669. doi:10.111/nph.12235

Lange, M., Habekost, M., Eisenhauer, N., Roscher, C., Bessler, H., Engels, C., Oelmann, Y., Scheu, S., Wilcke, W., Schulze, E., 2014. Biotic and Abiotic Properties Mediating Plant Diversity Effects on Soil Microbial Communities in an Experimental Grassland 9. doi:10.1371/journal.pone.0096182

Lynch, J.M., Whipps, J.M., 1990. Substrate flow in the rhizosphere. Plant and Soil 129, 1–10. doi:10.1007/BF00011685

Maarastawi, S.A., Frindte, K., Linnartz, M., Knief, C., 2018. Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of *Zea mays*. Frontiers in Microbiology. doi:10.3389/fmicb.2018.01295

Morriën, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zweers, H., De Hollander, M., Soto, R.L., Bouffaud, M.L., Buée, M., Dimmers, W., Duyts, H., Geisen, S., Girlanda, M., Griffiths, R.I., Jørgensen, H.B., Jensen, J., Plassart, P., Redecker, D., Schmelz, R.M., Schmidt, O., Thomson, B.C., Tisserant, E., Uroz, S., Winding, A., Bailey, M.J., Bonkowski, M., Faber, J.H., Martin, F., Lemanceau, P., De Boer, W., Van Veen, J.A., Van Der Putten, W.H., 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. Nature Communications 8. doi:10.1038/ncomms14349

Nakamura, A., Tun, C.C., Asakawa, S., Kimura, M., 2003. Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipid fatty acid analysis. Biology and Fertility of Soils 38, 288–295. doi:10.1007/s00374-003-0658-6

Peiffer, J.A., Spor, A., Koren, O., Jin, Z., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E., 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proceedings of the National Academy of Sciences of the United States of America 110, 6548–6553. doi:10.1073/pnas.1302837110

Plénet, D., Mollier, A., Pellerin, S., 2000. Growth analysis of maize field crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. Plant and Soil 224, 259–272. doi:10.1023/A:1004835621371

Schilling, G., Gransee, A., Deuhel, A., Ležoviž, G., Ruppel, S., 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Zeitschrift Für Pflanzenernährung Und Bodenkunde 161, 465–478. doi:10.1002/jpln.1998.3581610413

Swinnen, J., Van Veen, J.A., Merckx, R., 1994. Rhizosphere carbon fluxes in field-grown spring wheat: Model calculations based on 14C partitioning after pulse-labelling. Soil Biology and Biochemistry 26, 171–182. doi:10.1016/0038-0717(94)90160-0

van Veen, J.A., Liljeroth, E., Lekkerkerk, L.J.A., van de Geijn, S.C., 1991. Carbon fluxes in plant-soil systems at alevated atmospheric CO_2 levels. Ecological Applications 1, 175–181. doi:10.2307/1941810

Willers, C., Jansen van Rensburg, P.J., Claassens, S., 2015. Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. Journal of

Applied Microbiology 119, 1207–1218. doi:10.1111/jam.12902

3. Overall discussion

Traditional rice cultivation under flooded conditions is a major source of the global anthropogenic methane emissions (Cheng et al., 2013). The methane emissions from rice fields are linked to the physical, chemical and biological characteristics of flooded soils, which provide suitable niches for methane producing methanogenic archaea as well as for methane oxidizing bacteria (Wassmann et al., 2000a). Moreover, the cultivation of rice under flooded conditions leads to huge water consumption (Bouman et al., 2007; Tuong et al., 2005). To preserve water and minimize the release of methane, an increased number of farmers shift to a crop rotation system with rice cultivation in the wet season and maize cultivation in the dry season. The impact of introducing a crop rotation between rice and maize was investigated in the first phase of the multidisciplinary research project "Introduction of non-flooded crops in rice-dominated landscapes and its impact on carbon, nitrogen and water cycles (ICON)". It was observed that the seasonal change between wet and dry conditions in soils leads to a formation of desiccation cracks during maize cultivation, which causes loss of water, dissolved organic carbon and a greater release of the greenhouse gas N_2O (He et al., 2015; Weller et al., 2015, 2016). However, numerous questions remain open, especially about the biogeochemistry during the maize cultivation period in the dry season. As a second agricultural strategy, rice straw was introduced to compensate for the nutrient and water loss after drainage of the rice fields, because straw is known to reduce the evaporation and to contribute to the total supply of organic matter (Cabangon and Tuong, 2000; Kimura et al., 2004).

To investigate whether methanotrophs can inhabit the rice phyllosphere and therefore can be a potential sink for methane in flooded fields, a cultivation-dependent approach was used to enrich methanotrophic bacteria (**manuscript 1**). Microbial communities inhabiting the bulk soil and rhizosphere in paddy fields are well known, but knowledge about the influence of crop rotation on the microbial community is scarce. Therefore, the analysis microbial communities inhabiting drained rice fields under maize cultivation was mandatory to explore significant changes in comparison to monoculture rice and maize soils due to crop rotation (**manuscript 2**). Straw application was introduced as a further agricultural management strategy to prevent high water loss due to crack formation during maize cultivation. First, a comparative analysis of the impact of straw addition on bacterial and fungal communities in the bulk soil and rhizosphere of maize from soils with different management histories (rice monoculture, maize monoculture and rice-maize crop rotation (MR) soil) was conducted (**manuscript 2**). The active straw degrading community was identified in more detail in a paddy soil undergoing rice-maize crop rotation (**manuscript 3**). Furthermore, the influence of straw on the root exudate

consuming microbiota in the rhizosphere of maize was investigated in more detail during different developmental stages of the plant (**manuscript 4**).

3.1. Crop rotation

Despite the environmental problems that occur due to rice cultivation, the advantage of the highest possible yield and therefore the possibility to meet the increasing demand for rice is the reason to continue the cultivation of rice under flooded conditions (Conrad, 2007). This creates several anoxic niches in rice fields, which provide habitats, e.g. for methanogenic archaea that produce methane as end product of the anaerobic degradation of organic matter (Breidenbach and Conrad, 2015). Due to the methane production, rice fields provide an ideal habitat for methanotrophic bacteria utilizing methane as energy and carbon source and therefore acting as methane sink. Methanotrophs live at the interfaces between anoxic and oxic zones and attenuate the methane emissions of rice fields by up to 40 % (Conrad, 2007, 2009). Furthermore, the rice phyllosphere can serve as habitat for methanotrophs. Methanotrophs, which are living in association with rice plants, can use methane released from the water phase of flooded rice fields and by the rice plant itself, because of the ability of rice to passively transport methane to the atmosphere through its aerenchym. Previous studies identified type-I as well as type-II methanotrophs in the phyllosphere of rice with cultivation-independent studies (Iguchi et al., 2012; Knief et al., 2012). To test whether methanotrophic bacteria can be successfully isolated from aboveground plant parts, rice stems from monoculture rice fields were used for selective cultivation experiments. Methanotrophic bacteria belonging to type-I methanotrophs were successfully isolated (Frindte et al., 2017; **manuscript 1**). Interestingly, a representative of the previously uncultivated rice paddy cluster (RPC1) and a new strain of *Methylomagnum ishizawai* were described. This cluster is found in aquatic ecosystems and rice fields from various geographical origins (Knief, 2015). *PmoA* sequences are usually derived from the bulk soil or the rhizosphere of rice fields (Knief, 2015; Lüke et al., 2010). This leads to the assumption that the newly described representative of RPC1, *Methyloterricola oryzae,* and the new *Methylomagnum ishizawai* strain could possibly oxidize methane at the aboveground plant parts, which would represent a seldom regarded methane sink in monoculture rice fields.

Anyway, the activity of methanotrophs cannot fully mitigate the methane emissions, so that rice monoculture accounts for about 10 % of all anthropogenic methane emissions (Nazaries et al., 2013). Therefore, other strategies have to be developed, which still meet the increasing demand for stable food supply. Asian farmers use a crop rotation system between rice and upland crops to diminish water consumption (Bouman et al., 2005). In the Philippines, farmers usually cultivate maize as upland crop in the dry season, because of the simultaneous increasing demand for maize for poultry fattening and biofuel production (Bertomeu, 2012; Weller et al., 2015). The alteration between flooded and non-flooded conditions also results in reduced

methane emissions compared to flooded rice fields. The impact of the introduction of an upland crop like maize in rice soils on the inhabiting microbial communities is rarely studied and mostly focused on one compartment, i. e. either bulk soil or, to a less extent, the rhizosphere, and either on the bacterial or on the fungal community.

Concerning the effects of crop rotation, the main results of this thesis were that two major factors contribute to crop rotation dependent differences in microbial and fungal community composition: i) the altered oxygen availabilities in the different soils induced by the regular flooding of rice monoculture and rice-maize crop rotation soils, which leads to anaerobic conditions in soil, whereas in a maize monoculture the aerobic conditions remained stable and ii) the influence of crops and their specific rhizodeposits. Because of these factors, the long term maize and rice monoculture managed soils developed substantial differences in the microbial community composition. In monoculture soils, I observed common rice field or maize field soil bacterial and fungal taxa, respectively, as well as host-plant specific beneficial and pathogenic fungi. In comparison to those strong differences, the variation of the microbial communities between rice monoculture soils and MR soils was rather weak (**manuscript 2**). In previous studies, it was also detected that continuous crop rotation between paddy rice and maize, alfalfa or winter-wheat does not change the structure of the microbial community drastically (Breidenbach et al., 2015; Breidenbach and Conrad, 2015; Lopes et al., 2014; Zhao et al., 2014). Obviously, the temporally elongated drainage phase in crop rotation soils has only little impact on the microbial communities.

The higher similarity of the microbial community in MR soils to rice monoculture than to maize monoculture is due to the maintenance of an anaerobic microbial community in rice-maize crop rotation soils, even during maize cultivation (**manuscript 2**). The maintenance of an anaerobic community was also observed in a study investigating microbial communities in paddy soils, which were under crop rotation with alfalfa (Lopes et al., 2014). Likewise, Zhao et al. (2014) observed a higher relative abundance of anaerobic bacteria in soils of winter wheat-rice crop rotation than in soils of winter wheat-maize crop rotation. Even when paddy rice was introduced in an upland system (pasture) for the first time, an anaerobic microbial community established and remained stable during the pasture cultivation and even when the wetland conditions were followed by 4 years of upland conditions (Scavino et al., 2013). A stable methanogenic microbial community developed directly after the first introduction of paddy rice in the pasture system and tended to withstand periods of drainage once established (Scavino et al., 2013). The similarity of the microbiota inhabiting crop rotation soils to the microbiota that inhabits rice monoculture soils is promoted due to the previously mentioned regular changes between oxic

and anoxic conditions (Noll et al., 2005). Additionally, the paddy rice and MR soils in the Philippines that were investigated in this thesis have a high clay content and water holding capacity, which supports the maintenance of anaerobic micro-niches. Presumably, anaerobic microorganisms maintain their activity in these niches (Breidenbach et al., 2015; **manuscript 2**).

Nevertheless, the detected differences in the microbial communities between rice monoculture and the MR soil were mainly due to the reduction of anaerobic microorganisms in the MR soils (Maarastawi et al., 2018; **manuscript 2**). Liu et al. (2018) observed that methanogens persisted during the cultivation of upland crops in a paddy system, but their activity drastically decreased. This is caused due to the longer dry period and, therefore, higher oxygen availability in those soils, which causes oxygen stress for the soil inhabiting anaerobic microorganisms. Several previous studies detected also a decrease of anaerobic bacteria and archaea, especially methanogens, by introducing a crop rotation with different upland crops in a paddy soil system (Breidenbach et al., 2015; Breidenbach and Conrad, 2015; Liu et al., 2015; Lopes et al., 2014).

The smaller numbers of methanogens in crop rotation soils compared to rice soils indicates that crop rotation between rice and maize can be expected to be more ecologically friendly, because the main producers of methane are depleted or are suppressed. In agreement, reduced methane emissions in the same MR systems was observed previously (Weller et al., 2015, 2016). Less methane production in crop rotation soils also leads to a decrease of methanotrophs in comparison to rice monocultures (**manuscript 2**).

Besides the reduction of anaerobic bacterial taxa, I also detected a reduction of specific fungal taxa in MR as well as in rice monoculture soils in comparison to maize soils. The temporally or constantly flooded and therefore anoxic conditions in MR and rice soils suppressed the colonisation and the special enrichment of fungi in these soils. The reduction of anaerobic bacteria and archaea and the depletion of fungi in MR soils strongly indicates that the change from wetland to upland conditions causes the main differences in microbial community composition.

In contrast, the plant type and their rhizodeposits play a less important role and causes minor changes in microbial community composition. Even over time, i.e. up to 43 days of plant development, stronger differences between the crop rotation regimes (rice monoculture, maize monoculture, rice-maize crop rotation) were observed than between the different compartments (**manuscript 2**). Obviously, the amount and blend of carbon compounds released by maize have an inferior impact on the rhizosphere microbiota than the rice-maize crop rotation regime,

going along with different oxygen availabilities in the soils. Nevertheless, more genera responded to crop rotation in bulk soil than in the rhizosphere (**manuscript 2**), indicating that rhizosphere organisms are additionally controlled by plant root released carbon and are therefore less influenced by crop rotation than bulk soil microorganisms.

Taken together, it was shown that the establishment of crop rotation between rice and maize in a rice monoculture did not lead to dramatic changes in fungal and bacterial communities, as it was observed between rice and maize monoculture soils. The observed minor changes are mostly explained by oxygen availability, which is limited in MR soil as well as in rice monoculture soil during the wet season, whereas the soil under maize monoculture is characterized by predominantly oxic conditions. Therefore, the communities of MR soils are more similar to those in rice monoculture soils than to those in maize monoculture soils. Consequently, the oxygen availability is the driving factor causing the reduction of anaerobic species in MR soils, resulting in the main differences observed between the rice monoculture and MR soils.

3.2. Straw degradation in crop rotation bulk soil

The introduction of maize in a flooded rice system is known to lead to desiccation cracks and, therefore, to nutrient loss during the dry season (He et al., 2015; Weller et al., 2015, 2016). High nutrient removal due to high-yielding maize further contributes to the high nutrient loss during maize cultivation (Timsina et al., 2010). To compensate these losses, an application of rice straw, which remains as left-over from rice cultivation, was introduced in the rice-maize crop rotation soils after drainage. The return of rice straw to the fields contributes to the supply of organic matter and additionally conserves the moisture in soils, if it is applied as mulch (Cabangon and Tuong, 2000; Kimura et al., 2004). The combustion of rice straw and use of the ashes to fertilise the fields is another common practise (Gadde et al., 2009). However, the combustion ends up in a further high CO_2 production, so that the application of chopped rice straw into the drained field could be more ecological friendly.

The degradation of rice straw has been intensively studied in paddy soils under rice cultivation. In these cultivation systems, rice straw application is a common fertilisation practice, because it was shown that the use of chemical fertilisers results in a decrease of soil fertility (Asari et al., 2007; Ponnamperuma, 1984). In previous studies, the researchers investigated the straw degradation process under anoxic conditions in rice soils and observed a change in the bacterial and archaeal community composition over time. They showed that anaerobic bacteria like *Clostridia* and methanogenic archaea like *Methanosarcina* are the major rice straw degraders.

The increased occurrence of methanogens caused an increase in methane emissions (Asari et al., 2007; Conrad et al., 2012a, 2012b; Lee et al., 2017; Rui et al., 2009; Shrestha et al., 2011). In contrast to the increased number of anaerobic taxa, the occurrence of aerobic straw degrading bacteria was reduced (Rui et al., 2009) and the fungal community was even less important for the straw degradation process in anoxic rice soils (Murase et al., 2006; Nakamura et al., 2003). Lee et al. (2011, 2012) observed also an increase of methanogens over time, although they investigated the straw degradation process in rice soils under oxic conditions, but they additionally found aerobic bacterial genera belonging to *Proteobacteria*, *Bacilli* and *Actinobacteria* as straw degraders.

In contrast to flooded soils, the degradation of straw in upland soils is mainly conducted by aerobic bacteria as well as fungi, resulting in a rapid increase of CO_2 flux and, additionally, a change in bacterial and fungal community composition in comparison to upland soils without straw (Bastian et al., 2009; Bernard et al., 2007; Cleveland et al., 2007; Fan et al., 2014). Although much is known about straw or plant residue degradation in non-flooded soils or monoculture flooded soils, the community involved in the rice straw degradation process in rice soils undergoing crop rotation with maize has not yet been investigated. Especially the application of rice straw in a drained paddy soil during maize cultivation and the influences this has on the microbial community composition were investigated in this thesis for the first time. The longer exposure of MR soils to oxic conditions in comparison to paddy soils is one of the main differences between these two cultivation practices. Based on our findings that the microbial community in crop rotation soils is mainly shaped by oxygen availability (**manuscript 2**), the changes between flooded and non-flooded conditions are assumed to have also a major impact on the straw degrading community. In soils with rice monoculture and MR soils, where the flooding of soils is a reoccurring event, the impact of crop rotation and straw application was roughly equal (Maarastawi et al., 2018; **manuscript 2)**. These results confirm our previous assumption that oxygen is the main factor that influences the microbial community. Nevertheless, an impact of straw was evident in all investigated soils, indicating that straw addition leads to a shift in the microbial community composition in soils, regardless of the cultivation practice (rice or maize monoculture and MR soil). A previous study showed that changes of the microbial community composition due to straw application are correlated with the total abundance of carbon degradation genes and, therefore, affect the quantity and quality of carbon turnover in the soil (Zhao et al., 2016).

A stronger impact of straw addition was observed on the fungal than on the bacterial community composition in all investigated soils. Remarkably, the number of fungal genera that were

identified as significantly enriched was higher in treatments without straw than in treatments with straw (**manuscript 2**). This finding was confirmed by an analysis of the active straw degrading community in an experiment with labelled rice straw, where I observed that fungi were less involved in straw degradation than bacteria in MR soil (**manuscript 3**). The frequently flooded and, thus, anoxic conditions in MR soils seem to inhibit the degradation potential of fungi, likewise as observed in flooded rice fields under monoculture (Nakamura et al., 2003). A reason for that might be that straw degrading fungi are mostly aerobic, like the detected straw degraders *Cladorrhinum*, *Pestalotiopsis* and *Piriformospora* (Gasoni and Stegman de Gurfinkel, 1997; Qiang et al., 2012; Strobel et al., 1996; **manuscript 3**). This assumption is in agreement with the findings of Murase et al. (2012), who detected that fungi play a predominant role in the decomposition of fresh organic matter in rice soil under oxic conditions.

Compared to the fungal community, the bacterial community showed a different response to the application of straw. It resulted in an increase of bacterial diversity over time and in a special enrichment of diverse bacterial genera in all different soils (**manuscript 2, 3**). Such a development was also observed in other paddy soil studies performed under oxic as well as under anoxic conditions (Cleveland et al., 2007; Lee et al., 2017). The main bacterial straw degraders found in MR soils were similar to those in upland soils, i.e. genera of *Proteobacteria*, *Actinobacteria* and *Bacilli* (Bastian et al., 2009; Bernard et al., 2007; Cleveland et al., 2007; **manuscript 2, 3**). This is remarkable, because the results of **manuscript 2** showed that MR soils harbour bacterial communities more similar to flooded soils than to non-flooded soils. However, the main differences observed between microbial communities in rice monoculture and MR soils occurred due to a depletion of methanogens and *Clostridia* in MR soils (**manuscript 2**). These taxa are known to be the main straw degraders in flooded rice fields and were not among the active straw degraders in the investigated MR soil (**manuscript 3**). This finding is in line with the extended oxic phase in MR soil due to the maize cultivation period and indicates that the straw degrading microbial community was determined by the applied water regime in paddy fields. Our results showed that the degradation process of straw in a rice-maize crop rotation soil was mainly conducted by aerobic taxa, although previous studies showed that the degradation of straw in rice soils is mainly conducted by anaerobic bacteria and methanogenic archaea (Conrad et al., 2012a; Shrestha et al., 2011; Yuan et al., 2014). The absence of methanogens in fields managed under rice-maize crop rotation and treated with straw in comparison to rice fields leads to decreased methane production and emissions.

The straw degradation process is characterized by a temporal succession in upland soils, as well as in drained and flooded rice soils (Bernard et al., 2007; Lee et al., 2011, 2017; Rui et al., 2009; Sugano et al., 2005). This temporal dynamic is characterized by the degradation of easy degradable carbon compounds in an initial phase and the degradation of more complex carbon compounds in the later phase (Bernard et al., 2007; Lee et al., 2011; Peng et al., 2008; Rui et al., 2009; **manuscript 3**). This temporal succession was also seen in MR soils, with an immediate response of bacterial taxa and a delayed response of fungi to straw additions, indicating different lifestyles (copiotrophic vs. oligotrophic) of the straw degrading bacteria and fungi (**manuscript 3**). As different microbial communities are involved in the straw degradation process in the different soils, it is obvious that the phases of degradation are accomplished by different microbes in rice, upland or MR soils. In MR soils as well as in upland soils, aerobic copiotrophic bacteria like members of the classes *Alpha-, Beta-* and *Gammaproteobacteria* utilize easy-to-degrade straw compounds and immediately respond upon straw addition (Bernard et al., 2007; Fierer et al., 2007, **manuscript 3)**. In the later phase, oligotrophic members of *Actinobacteria* as well as members of *Deltaproteobacteria* and in particular several fungal taxa were involved in the straw degradation process in the MR soil, likewise as observed in other upland soils (Fan et al., 2014; Tardy et al., 2015; **manuscript 3**).

In paddy soils the main straw degraders in the early phase are anaerobic members of the clostridial clusters I and III (Lee et al., 2017; Rui et al., 2009), which are not involved in straw degradation in upland soils and the MR soil studied here (Bernard et al., 2007; **manuscript 3**). The degradation of easy-to-degrade compounds in paddy soils causes a rapid accumulation of fatty acids and acetate (Rui et al., 2009). This accumulation leads to a stimulation of methanogens during the later phase, so that a burst of methane production occurs. During the late succession, the gradual depletion of acetate results in a slowdown of methane production and a decrease of methanogens in paddy soils (Peng et al., 2008; Rui et al., 2009). During this phase, other groups of *Clostridia* (like clostridial cluster XIVa) as well as members of *Bacteroidetes*, *Chlorobi*, and *Actinobacteria* are involved in the straw degradation process in paddy soils (Lee et al., 2017; Rui et al., 2009). In contrast to MR and upland soils, fungi were not found to be involved in the straw degradation process in paddy soils, as they are suppressed by anoxic conditions. Only in rice soils incubated under oxic conditions, straw degrading fungi could be observed previously (Nakamura et al., 2003; Sugano et al., 2005). The main difference between the straw degradation process in MR or upland soils compared to paddy soils is that in MR and in upland soils aerobic microorganisms are responsible for the degradation in the early and in the late phase, whereas in rice fields the straw degradation is mainly conducted by anaerobic bacteria and methanogens in both phases.

In general, the effect of straw application on the microbial community residing in the MR soil was rather weak, only specific members responded to straw addition. Despite the weak impact on the microbiota, this process is important, because straw addition increases soil fertility and the water holding capacity (Cabangon and Tuong, 2000). The studies described in this thesis demonstrate that the whole straw decomposition process in MR soils is conducted by aerobic microorganisms and differs in that aspect from straw degradation in paddy soils under rice monoculture. In rice monoculture soils mainly anaerobic bacteria and methanogens are involved in straw degradation, whereas in MR soils a depletion of anaerobic bacteria and methanogens involved in the straw degradation was observed. Therefore, straw application in MR soils does not only lead to a soil fertilising effect, but also does not contribute to an increase in methane emissions as it is known for the straw application in paddy soils. The straw decomposition in MR soils showed a clear temporal succession with an instantaneous response of aerobic bacteria and a delayed response of fungi. Especially the copiotrophic bacteria decompose easy to degrade carbon compounds in the early phase, while oligotrophic fungi degrade predominantly the more complex carbon compounds in a later phase.

3.3. Straw degradation in the rhizosphere

In comparison to bulk soil, the rhizosphere is characterized by a continuous carbon input through the release of rhizodeposits (Bowen and Rovira, 1999; Peiffer et al., 2013). Especially root exudates are of prime importance for microorganisms, since root exudates are readily assimilable and therefore create a hot spot of microbial activity (Baudoin et al., 2003). Root exudates stimulate growth and lead to distinct microbial communities in the rhizosphere compared to the bulk soil (Baudoin et al., 2003).

Recent studies on straw application investigated the effect on the microbial community composition focusing on soils with the same cultivation history (e.g. flooded or non-flooded), either in the rhizosphere (Peng et al., 2016; Shrestha et al., 2011) or in bulk soil (Conrad et al., 2012a; Lee et al., 2017; Rui et al., 2009). However, a direct comparison of the straw degrading process and the involved microbes in bulk soil versus rhizosphere of crop rotation soil was lacking so far. Furthermore, it is unknown whether and to what extent the rhizosphere microbiota uses additional carbon sources like straw.

In soils treated with straw a comparison of microbial communities between bulk soil and rhizosphere showed that straw induced a stronger change in rhizosphere communities than in bulk soil (**manuscript 2**). This was not necessarily expected because the rhizosphere microbiota is considered to be mainly influenced by root exudation (Berg and Smalla, 2009; Bulgarelli et

al., 2013). The detailed analysis of the active straw degrading community with ^{13}C-labelled rice straw in the MR soil showed a detection of specially labelled microbial taxa in the rhizosphere. This suggests that a subpopulation of the maize rhizosphere microbiota benefited from straw addition (**manuscript 3**). Surprisingly, several taxa of this subpopulation were not involved in straw degradation in the bulk soil and were not even detectable there. The appearance of those taxa being exclusively labelled in the rhizosphere induced the strong change in microbial community composition due to straw addition in comparison to bulk soil (**manuscript 2, 3**).

Most of the straw degrading taxa that were only found in the rhizosphere of maize but not in bulk soil were previously detected in the rhizosphere of different plants (Jilani et al., 2007; Naz et al., 2014; Smalla et al., 2001; Yang and Crowley, 2000). The detection of exclusively straw degrading taxa in the rhizosphere and the detection of taxa, which were detected in rhizosphere samples before, indicate that these organisms are able to use root exudates as well as carbon derived from additional sources like straw.

In general the straw degrading microorganisms, which I found in these studies in bulk soil and rhizosphere of MR soil, were mainly aerobic bacteria and fungi. Additionally, the taxa that where exclusively labelled in the rhizosphere but not in bulk soil were mainly detected before in the rhizosphere of plants, which are cultivated in upland soils. Only *Roseomonas* was isolated before from the rhizosphere of rice in a flooded soil (Chung et al., 2015; Jilani et al., 2007; Naz et al., 2014; Smalla et al., 2001; Yang and Crowley, 2000). The predominant appearance of aerobic organisms as straw degraders showed that the straw degrading community of the maize rhizosphere in MR soil is more alike to the community found in oxic soils than in anoxic soils. This trend was also observed in the work of this thesis for the straw degrading community in the bulk soil (**manuscript 3)** as discussed before. In contrast, Shrestha et al. (2011) analysed the straw degrading community in the rhizosphere of flooded rice and observed an increase of mostly anaerobic microbes, e.g. methanogens and *Clostridia*.

Although the straw application induced a stronger community shift and a higher diversity in the rhizosphere, which can be explained by the stimulation of specialized straw degraders, the utilization of straw in bulk soil was more intensive (**manuscript 2, 3**). This was shown by a higher number of labelled taxa and higher label intensity in bulk soil samples (**manuscript 3**). As the rhizosphere is a hotspot for microbial activity, induced by the continuous carbon input, rhizosphere microorganisms are not necessarily reliant on an additional carbon source. Furthermore, the rhizodeposits are easier bioavailable than plant residues and therefore preferentially used (Kuzyakov, 2010). Both, the less intensive use of straw in the rhizosphere and the detection of some taxa, which degrade straw preferentially in the rhizosphere compared

to the bulk soil, indicate a synergistic effect in the rhizosphere, i. e. the use of root exudates and straw. Previously, Kuzyakov et al. (2007) showed a similar effect by detecting a small but significant increase in litter decomposition after adding easy degradable root exudates to a rhizosphere model system.

I additionally investigated the effect of straw addition on active exudate-using microbes by labelling maize plants with $^{13}CO_2$ (**manuscript 4**). Thereby a stronger utilization of root exudates in the presence of straw than in the absence was observed 20 days after maize seeding **(manuscript 4).** After this time of incubation, I detected a predominant straw degradation by oligotrophic organisms in **manuscript 3**, because more complex straw compounds were available at this time. Transferred to the results of **manuscript 4**, I conclude that the strong utilization of root exudates 20 days after maize seeding in treatments with straw was due to the fact that mainly complex straw compounds were available and these compounds could only be degraded by a few oligotrophic organisms. Therefore, most rhizosphere organisms use root exudates as carbon source (**manuscript 4**) and just a few oligotrophic rhizosphere microorganisms use straw as an additional source 20 days after seeding of maize. This is in agreement with the lower incorporation of carbon derived from straw by rhizosphere microorganisms than by bulk soil organisms after 20 days, which I observed in the experiment with ^{13}C-labelled straw (**manuscript 3**). The degradation of complex compounds by microorganisms leads to the release of more easily-available carbon compounds derived from straw (De Boer et al., 2005), so that rhizosphere organisms may partly switch their carbon source utilization pattern and use relatively more straw instead of root exudates at later time points. In agreement, I detected a lower use of root exudates by microbes in treatments with straw than without straw in the rhizosphere of 30 and 40 day old maize plants (**manuscript 4**). This is in agreement with the observations in the bulk soil of maize in **manuscript 3**. There I detected a second increase of bacterial label intensity due to straw addition after 35 days of incubation, which indicates that the bacteria could use the released easy-available straw compounds after this time of incubation. Taken together, these observations show that straw addition influences the uptake of root exudates. I suggest that this effect depends on the temporal succession of straw decomposition, which was also observed in bulk soil of MR soil (**manuscript 3**). This means that the temporal succession of straw decomposition in the rhizosphere is similar to that in bulk soil.

Altogether, it was shown that the microbial community of the rhizosphere is more strongly affected by straw addition than the bulk soil inhabiting community, due to a straw degrading community that exclusively occurs in the rhizosphere. Several of the fungal and bacterial taxa

degrading straw detected at the investigated time point (20 days after maize seeding) formed a specific subpopulation in the rhizosphere and are known to have an oligotrophic lifestyle. Those are able to use recalcitrant straw compounds and can therefore outcompete the copiotrophic microorganisms in the rhizosphere. The simultaneous utilization of carbon derived from root exudates and straw affected the uptake of root exudates, but this effect was dependent on the stage of straw decomposition.

3.4. Final conclusion

The reduction of methane emissions and water consumption is an important goal to achieve a more environmental friendly rice cultivation practise in Asia. The introduction of a crop rotation system with rice in the wet season and maize in the dry season is a promising alternative to rice cultivation in monoculture under flooded conditions. Additionally, the generated rice straw can be incorporated into the soil and applied as mulch in the dry season. This causes a fertilising effect by returning carbon and nutrients to the soil and helps to conserve the moisture in soil. Therefore, crop rotation and straw application are management strategies that save resources (water and fertiliser) and furthermore reduce the global warming effect by decreased CH_4 emissions in comparison to the cultivation of rice in monoculture. Methane is mainly produced in flooded rice fields and despite the activity of methanotrophs in rice fields, the methane emissions of flooded rice fields are much higher than of MR or upland fields.

The extended drainage period in MR soils causes only little changes in microbial communities, because the regular change between oxic and anoxic conditions remains in both, rice monoculture and MR systems. Nevertheless, an important difference between the microbial communities in rice monoculture and MR soils is the suppression of anaerobic microorganisms (figure 3.1). The longer aeration period in soils under crop rotation compared to those under rice monoculture causes a suppression of the soil inhabiting anaerobic microorganisms, indicating that oxygen is the main driving factor for shaping the microbial community composition.

The degradation of straw in MR soil is mainly accomplished by aerobic bacteria in the bulk soil as well as in the rhizosphere. Additionally, fungi have a delayed response to straw addition. They are predominantly involved in the degradation of complex carbon compounds. In contrast, the degradation in the anoxic bulk soil and in the rhizosphere of rice is mainly conducted by anaerobic *Clostridia* and methanogenic archaea, and is characterized by the absence of fungi. The straw degradation process follows a temporal succession, in which different microbial taxa are involved over time. The decomposition stage of straw also influences the uptake of root

exudates by rhizosphere microorganisms, because some root exudate consuming microbes are able to use straw as additional carbon source.

In conclusion, the establishment of crop rotation in combination with straw addition is a promising strategy to reduce the methane emissions of paddy soils and, simultaneously, to preserve soil moisture and soil quality by straw addition, because the microorganisms responsible for methane production in paddy soils are depleted under these conditions. In MR soils treated with straw, no active straw degrading methanogens were detected, leading to the conclusion that despite straw addition no increase in methane production will occur, as it was found previously for rice soils treated with straw.

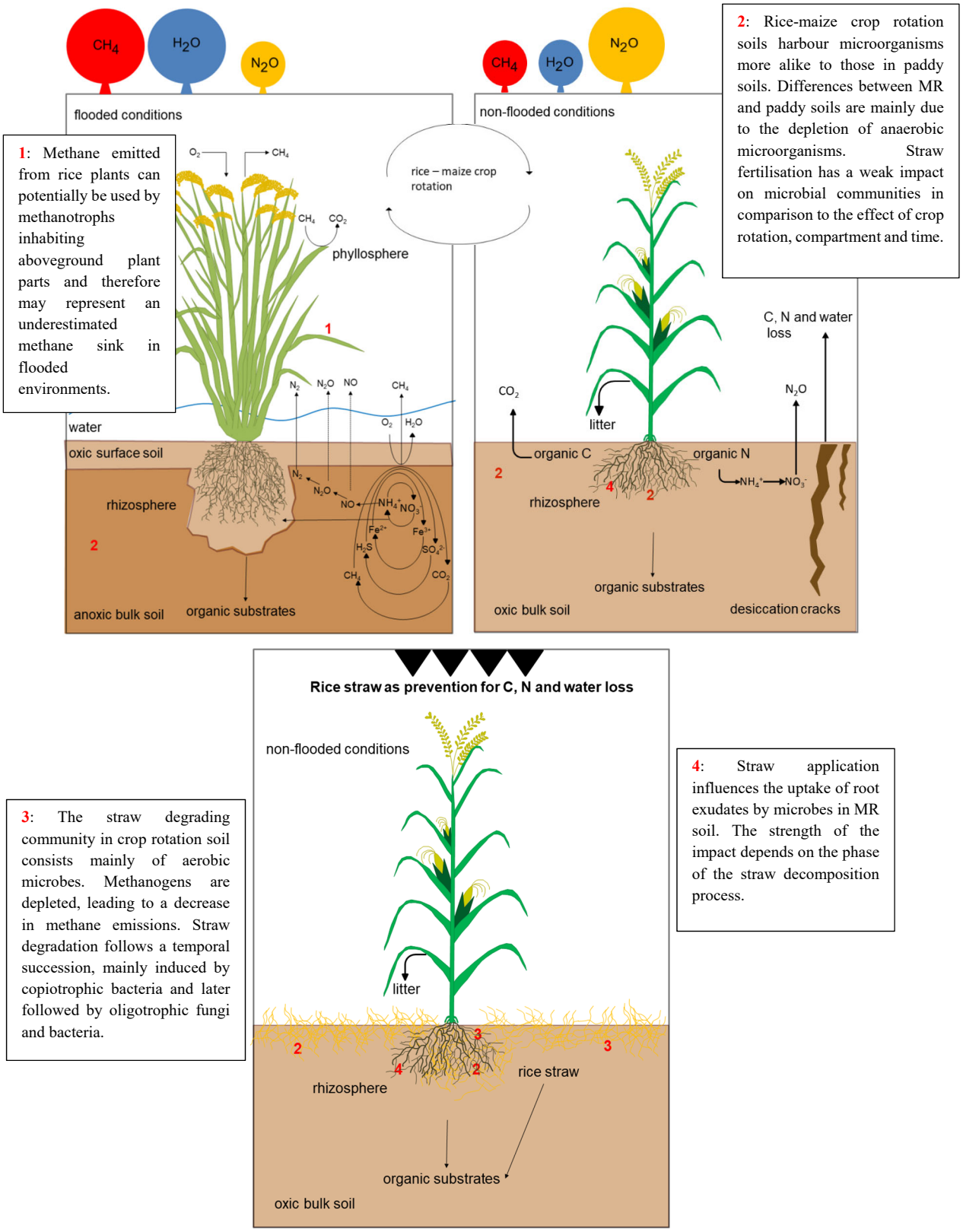


Figure 3.1: Graphical summary of main results achieved from the studies of this thesis.

4. References

Aira, M., Gómez-Brandón, M., Lazcano, C., Bååth, E., and Domínguez, J. (2010). Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil Biol. Biochem.* 42, 2276–2281. doi:10.1016/j.soilbio.2010.08.029.

Asari, N., Ishihara, R., Nakajima, Y., Kimura, M., and Asakawa, S. (2007). Succession and phylogenetic composition of eubacterial communities in rice straw during decomposition on the surface of paddy field soil. *Soil Sci. Plant Nutr.* 53, 56–65. doi:10.1111/j.1747-0765.2007.00110.x.

Badri, D. V., and Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell Environ.* 32, 666–681. doi:10.1111/j.1365-3040.2009.01926.x.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266. doi:10.1146/annurev.arplant.57.032905.105159.

Bastian, F., Bouziri, L., Nicolardot, B., and Ranjard, L. (2009). Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biol. Biochem.* 41, 262–275. doi:10.1016/j.soilbio.2008.10.024.

Baudoin, E., Benizri, E., and Guckert, a (2001). Metabolic structure of bacterial communities from district maize rhizosphere compartments. *Eur. J. Soil Biol.* 37, 85–93.

Baudoin, E., Benizri, E., and Guckert, A. (2002). Impact of growth stage on the bacteria community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Appl. Soil Ecol.* 19, 135–145.

Baudoin, E., Benizri, E., and Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biol. Biochem.* 35, 1183–1192. doi:10.1016/S0038-0717(03)00179-2.

Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* 68, 1–13. doi:10.1111/j.1574-6941.2009.00654.x.

Bernard, L., Mougel, C., Maron, P.-A., Nowak, V., Lévêque, J., Henault, C., et al. (2007). Dynamics and identification of soil microbial populations actively assimilating carbon from ^{13}C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. *Environ. Microbiol.* 9, 752–764. doi:10.1111/j.1462-2920.2006.01197.x.

Bertomeu, M. (2012). Growth and yield of maize and timber trees in smallholder agroforestry systems in Claveria , northern Mindanao , Philippines. 73–87. doi:10.1007/s10457-011-9444-x.

Bouman, B. A. M., Humphreys, E., Tuong, T. P., and Barker, R. (2007). Rice and Water. *Adv. Agron.* 92, 187–237. doi:10.1016/S0065-2113(04)92004-4.

Bouman, B. A. M., Peng, S., Castan, A. R., and Visperas, R. M. (2005). Yield and water use of irrigated tropical aerobic rice systems. 74, 87–105. doi:10.1016/j.agwat.2004.11.007.

Bowen, G. D., and Rovira, A. D. (1999). "The Rhizosphere and its management to improve plant growth," in Advances in Agronomy., ed. D. L. Sparks (Academic Press), 1–102. doi:https://doi.org/10.1016/S0065-2113(08)60425-3.

Breidenbach, B., Blaser, M. B., Klose, M., and Conrad, R. (2015). Crop rotation of flooded rice with upland maize impacts the resident and active methanogenic microbial community. *Environ. Microbiol.* 18, 2868–2885. doi:10.1111/1462-2920.13041.

Breidenbach, B., and Conrad, R. (2015). Seasonal dynamics of bacterial and archaeal methanogenic communities in flooded rice fields and effect of drainage. *Front. Microbiol.* 5, 1–16. doi:10.3389/fmicb.2014.00752.

Breidenbach, B., Pump, J., and Dumont, M. G. (2016). Microbial community structure in the rhizosphere of rice plants. *Front. Microbiol.* 6, 1–12. doi:10.3389/fmicb.2015.01537.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* 64, 807–838. doi:10.1146/annurev-arplant-050312-120106.

Butler, J. L., Williams, M. a, Bottomley, P. J., and Myrold, D. D. (2003). Microbial community dynamics associated with rhizosphere carbon flow microbial community dynamics associated with rhizosphere carbon flow. *Appl. Environ. Microbiol.* 69, 6793–6800. doi:10.1128/AEM.69.11.6793.

Cabangon, R. J., and Tuong, T. P. (2000). Management of cracked soils for water saving during land preparation for rice cultivation. *Soil Tillage Res.* 56, 105–116. doi:10.1016/S0167-1987(00)00125-2.

Castellanos, T., Dohrmann, A. B., Imfeld, G., Baumgarte, S., and Tebbe, C. C. (2009). Search of environmental descriptors to explain the variability of the bacterial diversity from maize rhizospheres across a regional scale. *Eur. J. Soil Biol.* 45, 383–393. doi:10.1016/j.ejsobi.2009.07.006.

Cheng, K., Ogle, S. M., Parton, W. J., and Pan, G. (2013). Predicting methanogenesis from rice paddies using the DAYCENT ecosystem model. *Ecol. Modell.* 261–262, 19–31. doi:10.1016/j.ecolmodel.2013.04.003.

Chung, E. J., Yoon, H. S., Kim, K. H., Jeon, C. O., and Chung, Y. R. (2015). *Roseomonas oryzicola* sp. Nov., isolated from the rhizosphere of rice (Oryza sativa L.). *Int. J. Syst. Evol. Microbiol.* 65, 4839–4844. doi:10.1099/ijsem.0.000656.

Cleveland, C. C., Nemergut, D. R., Schmidt, S. K., and Townsend, A. R. (2007). Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82, 229–240. doi:10.1007/s10533-006-9065-z.

Conrad, R. (2007). Microbial Ecology of Methanogens and Methanotrophs. *Adv. Agron.* 96, 1–63. doi:10.1016/S0065-2113(07)96005-8.

Conrad, R. (2009). The global methane cycle: Recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* 1, 285–292. doi:10.1111/j.1758-2229.2009.00038.x.

Conrad, R., and Frenzel, P. (2003). "Flooded Soils," in *Encyclopedia of Environmental Microbiology* (American Cancer Society). doi:10.1002/0471263397.env034.

Conrad, R., Klose, M., Lu, Y., and Chidthaisong, A. (2012a). Methanogenic pathway and archaeal communities in three different anoxic soils amended with rice straw and maize straw. *Front. Microbiol.* 3, 1–12. doi:10.3389/fmicb.2012.00004.

Conrad, R., Klose, M., Yuan, Q., Lu, Y., and Chidthaisong, A. (2012b). Stable carbon isotope fractionation, carbon flux partitioning and priming effects in anoxic soils during methanogenic degradation of straw and soil organic matter. *Soil Biol. Biochem.* 49, 193–199. doi:10.1016/j.soilbio.2012.02.030.

Davidson, E. a., Keller, M., Erickson, H. E., Verchot, L. V., and Veldkamp, E. (2000). Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides. *Bioscience* 50, 667. doi:10.1641/0006-3568(2000)050[0667:TACMOS]2.0.CO;2.

De Boer, W., Folman, L. B., Summerbell, R. C., and Boddy, L. (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* 29, 795–811. doi:10.1016/j.femsre.2004.11.005.

Derrien, D., Marol, C., and Balesdent, J. (2005). The dynamics of neutral sugars in the rhizosphere of wheat. An approach by 13C pulse-labelling and GC/C/IRMS. *Plant Soil* 267, 243–253. doi:10.1007/s11104-005-5348-8.

España, M., Rasche, F., Kandeler, E., Brune, T., Rodriguez, B., Bending, G. D., et al. (2011). Assessing the effect of organic residue quality on active decomposing fungi in a tropical Vertisol using15N-DNA stable isotope probing. *Fungal Ecol.* 4, 115–119. doi:10.1016/j.funeco.2010.09.005.

Fan, F., Yin, C., Tang, Y., Li, Z., Song, A., Wakelin, S. A., et al. (2014). Probing potential microbial coupling of carbon and nitrogen cycling during decomposition of maize residue by ^{13}C-DNA-SIP. *Soil Biol. Biochem.* 70, 12–21. doi:10.1016/j.soilbio.2013.12.002.

Fierer, N., Bradford, M. A. A., and Jackson, R. B. B. (2007). Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. doi:10.1890/05-1839.

Fontaine, S., Mariotti, A., and Abbadie, L. (2003). The priming effect of organic matter: A question of microbial competition? *Soil Biol. Biochem.* 35, 837–843. doi:10.1016/S0038-0717(03)00123-8.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., et al. (2007). Changes in atmospheric constituents and in radiative forcing (IPCC 2007). *Clim. Chang. 2007 Phys. Sci. Basis. Contrib. Work. Gr. I to Fourth Assess. Rep. Intergov. Panel Clim. Chang.* 30, 129–234. doi:10.1103/PhysRevB.77.220407.

Frindte, K., Maarastawi, S. A., Lipski, A., Hamacher, J., and Knief, C. (2017). Characterization of the first rice paddy cluster I isolate, Methyloterricola oryzae gen. nov., sp. nov. and amended description of Methylomagnum ishizawai. *Int. J. Syst. Evol. Microbiol.* 67, 4507–4514. doi:10.1099/ijsem.0.002319.

Fuhrmann, I., He, Y., Lehndorff, E., Brüggemann, N., Amelung, W., Wassmann, R., et al. (2018). Nitrogen fertilizer fate after introducing maize and upland-rice into continuous paddy rice cropping systems. *Agric. Ecosyst. Environ.* 258, 162–171. doi:10.1016/j.agee.2018.02.021.

Gadde, B., Bonnet, S., Menke, C., and Garivait, S. (2009). Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. *Environ. Pollut.* 157, 1554–1558. doi:https://doi.org/10.1016/j.envpol.2009.01.004.

Gasoni, L., and Stegman de Gurfinkel, B. (1997). The endophyte *Cladorrhinum foecundissimum*in cotton roots: phosphorus uptake and host growth. *Mycol. Res.* 101, 867–870. doi:DOI: undefined.

Glissmann, K., and Conrad, R. (2002). Saccharolytic activity and its role as a limiting step in methane formation during the anaerobic degradation of rice straw in rice paddy soil. *Biol. Fertil. Soils* 35, 62–67. doi:10.1007/s00374-002-0442-z.

Haichar, F. el Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* 2, 1221–1230. doi:10.1038/ismej.2008.80.

Haichar, F. el Z., Santaella, C., Heulin, T., and Achouak, W. (2014). Root exudates mediated interactions belowground. *Soil Biol. Biochem.* 77, 69–80. doi:10.1016/j.soilbio.2014.06.017.

He, Y., Siemens, J., Amelung, W., Goldbach, H., Wassmann, R., Alberto, M. C. R., et al. (2015). Carbon release from rice roots under paddy rice and maize-paddy rice cropping. *Agric. Ecosyst. Environ.* 210, 15–24. doi:10.1016/j.agee.2015.04.029.

Heyer, J., Galchenko, V. F., and Dunfield, P. F. (2002). Molecular phylogeny of type II methane- oxidizing bacteria isolated from various enviroments. 2831–2846.

Iguchi, H., Sato, I., Sakaibara, M., Yurimoto, H., and Sakai, Y. (2012). Distribution of Methanotrophs in the Phyllosphere. *Biosci. Biotechnol. Biochem.* 76, 1580–1583. doi:10.1271/bbb.120281.

Jilani, G., Akram, A., Ali, R. M., Hafeez, F. Y., Shamsi, I. H., Chaudhry, A. N., et al. (2007). Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. *Ann. Microbiol.* 57, 177–184. doi:10.1007/BF03175204.

Jones, D. L., Nguyen, C., and Finlay, R. D. (2009). Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. *Plant Soil* 321, 5–33. doi:10.1007/s11104-009-9925-0.

Kimura, M., Miyaki, M., Fujinaka, K. I., and Maie, N. (2001). Microbiota responsible for the decomposition of rice straw in a submerged paddy soil estimated from phospholipid fatty acid composition. *Soil Sci. Plant Nutr.* 47, 569–578. doi:10.1080/00380768.2001.10408420.

Kimura, M., Murase, J., and Lu, Y. (2004). Carbon cycling in rice field ecosystems in the context of input, decomposition and translocation of organic materials and the fates of their end products (CO_2 and CH_4). *Soil Biol. Biochem.* 36, 1399–1416. doi:10.1016/j.soilbio.2004.03.006.

Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Front. Microbiol.* 6. doi:10.3389/fmicb.2015.01346.

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., et al. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *ISME J.* 6, 1378–1390. doi:10.1038/ismej.2011.192.

Kool, D. M., Dolfing, J., Wrage, N., and Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biol. Biochem.* 43, 174–178. doi:10.1016/j.soilbio.2010.09.030.

Kusliene, G., Rasmussen, J., Kuzyakov, Y., and Eriksen, J. (2014). Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by C and ^{15}N labelled exudates. *Soil Biol. Biochem.* 76, 22–33. doi:10.1016/j.soilbio.2014.05.003.

Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. *Soil Biol. Biochem.* 42, 1363–1371. doi:10.1016/j.soilbio.2010.04.003.

Kuzyakov, Y., Hill, P. W., and Jones, D. L. (2007). Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. *Plant Soil* 290, 293–305. doi:10.1007/s11104-006-9162-8.

Lee, C. G., Watanabe, T., and Asakawa, S. (2017). Bacterial community incorporating carbon derived from plant residue in an anoxic non-rhizosphere soil estimated by DNA-SIP analysis. *J. Soils Sediments* 17, 1084–1091. doi:10.1007/s11368-016-1621-0.

Lee, C. G., Watanabe, T., Murase, J., Asakawa, S., and Kimura, M. (2012). Growth of methanogens in an oxic soil microcosm: Elucidation by a DNA-SIP experiment using ^{13}C-labeled dried rice callus. *Appl. Soil Ecol.* 58, 37–44. doi:10.1016/j.apsoil.2012.03.002.

Lee, C. G., Watanabe, T., Sato, Y., Murase, J., Asakawa, S., and Kimura, M. (2011). Bacterial populations assimilating carbon from ^{13}C-labeled plant residue in soil: Analysis by a DNA-SIP approach. *Soil Biol. Biochem.* 43, 814–822. doi:10.1016/j.soilbio.2010.12.016.

Li, X., Rui, J., Mao, Y., Yannarell, A., and Mackie, R. (2014). Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biol. Biochem.* 68, 392–401. doi:10.1016/j.soilbio.2013.10.017.

Liesack, W., Schnell, S., and Revsbech, N. P. (2000). Microbiology of fooded rice paddies. *FEMS* 24, 625–645. doi:doi.org/10.1111/j.1574-6976.2000.tb00563.x.

Liu, D., Ishikawa, H., and Nishida, M. (2015). Effect of paddy-upland rotation on methanogenic archaeal community structure in paddy field soil. *Microb. Ecol.* 69, 160–168. doi:10.1007/s00248-014-0477-3.

Liu, D., Nishida, M., Takahashi, T., and Asakawa, S. (2018). Transcription of mcrA gene decreases upon prolonged non-flooding period in a methanogenic archaeal community of a paddy-upland rotational field soil. 751–760. doi:10.1007/s00248-017-1063-2.

Lopes, A. R., Manaia, C. M., and Nunes, O. C. (2014). Bacterial community variations in an alfalfa-rice rotation system revealed by 16S rRNA gene 454-pyrosequencing. *FEMS Microbiol. Ecol.* 87, 650–663. doi:10.1111/1574-6941.12253.

Lu, Y., and Conrad, R. (2005). In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science (80-.).* 309, 1088–1090. doi:10.1126/science.1113435.

Lu, Y., Murase, J., Watanabe, A., Sugimoto, A., and Kimura, M. (2004). Linking microbial community dynamics to rhizosphere carbon flow in a wetland rice soil. *FEMS Microbiol. Ecol.* 48, 179–186. doi:10.1016/j.femsec.2004.01.004.

Lüke, C., Krause, S., Cavigiolo, S., Greppi, D., Lupotto, E., and Frenzel, P. (2010). Biogeography of wetland rice methanotrophs. *Environ. Microbiol.* 12, 862–872. doi:10.1111/j.1462-2920.2009.02131.x.

Lynch, J. M., and Whipps, J. M. (1990). Substrate flow in the rhizosphere. *Plant Soil* 129, 1–10. doi:10.1007/BF00011685.

Ma, K., Conrad, R., and Lu, Y. (2011). Responses of methanogen mcrA genes and their transcripts to alternate dry wet cycle of paddy field soil. doi:10.1128/AEM.06934-11.

Ma, K., Conrad, R., and Lu, Y. (2013). Dry/wet cycles change the activity and population dynamics of methanotrophs in rice field soil. *Appl. Environ. Microbiol.* 79, 4932–4939. doi:10.1128/AEM.00850-13.

Ma, K., and Lu, Y. (2011). Regulation of microbial methane production and oxidation by intermittent drainage in rice field soil. *FEMS Microbiol. Ecol.* 75, 446–456. doi:10.1111/j.1574-6941.2010.01018.x.

Maarastawi, S. A., Frindte, K., Linnartz, M., and Knief, C. (2018). Crop rotation and straw application impact microbial communities in Italian and Philippine soils and the rhizosphere of *Zea mays*. *Front. Microbiol.* doi:10.3389/fmicb.2018.01295.

Megonigal, J. P., and Guenther, A. B. (2008). Methane emissions from upland forest soils and vegetation. *Tree Physiol.* 28, 491–498. doi:10.1093/treephys/28.4.491.

Murase, J., Matsui, Y., Katoh, M., Sugimoto, A., and Kimura, M. (2006). Incorporation of ^{13}C-labeled rice-straw-derived carbon into microbial communities in submerged rice field soil and percolating water. *Soil Biol. Biochem.* 38, 3483–3491. doi:10.1016/j.soilbio.2006.06.005.

Murase, J., Shibata, M., Lee, C. G., Watanabe, T., Asakawa, S., and Kimura, M. (2012). Incorporation of plant residue-derived carbon into the microeukaryotic community in a rice field soil revealed by DNA stable-isotope probing. *FEMS Microbiol. Ecol.* 79, 371–379. doi:10.1111/j.1574-6941.2011.01224.x.

Na Bhadalung, N., Suwanarit, A., Dell, B., Nopamornbodi, O., Thamchaipenet, A., and Rungchuang, J. (2005). Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system. *Plant Soil* 270, 371–382. doi:10.1007/s11104-004-1829-4.

Nakamura, A., Tun, C. C., Asakawa, S., and Kimura, M. (2003). Microbial community responsible for the decomposition of rice straw in a paddy field: Estimation by phospholipid fatty acid analysis. *Biol. Fertil. Soils* 38, 288–295. doi:10.1007/s00374-003-0658-6.

Naz, I., Mirza, M. S., and Bano, A. (2014). Molecular Characterization of Rhizosphere Bacterial Communities Associated With Wheat (*Triticum Aestivum* L.) Cultivars At. *JAPS, J. Anim. Plant ...* 24, 1123–1134. Available at: http://www.thejaps.org.pk/docs/v-24-4/23.pdf.

Nazaries, L., Murrell, J. C., Millard, P., Baggs, L., and Singh, B. K. (2013). Methane, microbes and models: Fundamental understanding of the soil methane cycle for future predictions. *Environ. Microbiol.* 15, 2395–2417. doi:10.1111/1462-2920.12149.

Nguyen, C. (2003). Rhizodeposition of organic C by plants: mechanisms and controls. *Agon. EDP Sci.* 23, 375–396. doi:10.1051/agro.

Noll, M., Matthies, D., Frenzel, P., Derakshani, M., and Liesack, W. (2005). Succession of bacterial community structure and diversity in a paddy soil oxygen gradient. *Environ. Microbiol.* 7, 382–395. doi:10.1111/j.1462-2920.2004.00700.x.

Ogiso, T., Ueno, C., Huy, T. Van, Katayama, A., Kimura, M., and Asakawa, S. (2012). Methylomonas koyamae sp . nov ., a type I methane-oxidizing bacterium from floodwater of a rice paddy field. 1832–1837. doi:10.1099/ijs.0.035261-0.

Parry, M. L., Rosenzweig, C., Iglesias, A., Livermore, M., and Fischer, G. (2004). Effects of climate change on global food production under SRES emissions and socio-economic scenarios. *Glob. Environ. Chang.* 14, 53–67. doi:10.1016/j.gloenvcha.2003.10.008.

Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., et al. (2013). Stimulation of Different Functional Groups of Bacteria by Various Plant Residues as a Driver of Soil Priming Effect. *Ecosystems* 16, 810–822. doi:10.1007/s10021-013-9650-7.

Pausch, J., and Kuzyakov, Y. (2011). Photoassimilate allocation and dynamics of hotspots in roots visualized by ^{14}C phosphor imaging. *J. Plant Nutr. Soil Sci.* 174, 12–19. doi:10.1002/jpln.200900271.

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., et al. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proc. Natl. Acad. Sci. U. S. A.* 110, 6548–6553. doi:10.1073/pnas.1302837110.

Peng, C., Lai, S., Luo, X., Lu, J., Huang, Q., and Chen, W. (2016). Environment effects of long term rice straw application on the microbial communities of rapeseed rhizosphere in a paddy-upland rotation system. *Sci. Total Environ.* 557–558, 231–239. doi:10.1016/j.scitotenv.2016.02.184.

Peng, J., Lü, Z., Rui, J., and Lu, Y. (2008). Dynamics of the methanogenic archaeal community during plant residue decomposition in an anoxic rice field soil. *Appl. Environ. Microbiol.* 74, 2894–2901. doi:10.1128/AEM.00070-08.

Ponnamperuma, F. N. (1984). Straw as a source of nutrient for wetland rice. *Org. Matter Rice*, 117–136.

Qiang, X., Weiss, M., Kogel, K. H., and Schäfer, P. (2012). *Piriformospora indica*-a mutualistic *basidiomycete* with an exceptionally large plant host range. *Mol. Plant Pathol.* 13, 508–518. doi:10.1111/j.1364-3703.2011.00764.x.

Rosegrant, M. (2008). Biofuels and grain prices: impacts and policy responses. *Int. Food Policy Res. Inst.*, 1–4. Available at: http://www.grid.unep.ch/FP2011/step1/pdf/004_Rosegrant_2008.pdf.

Rui, J., Peng, J., and Lu, Y. (2009). Succession of bacterial populations during plant residue decomposition in rice field soil. *Appl. Environ. Microbiol.* 75, 4879–4886. doi:10.1128/AEM.00702-09.

Scavino, A. F., Ji, Y., Pump, J., Klose, M., Claus, P., and Conrad, R. (2013). Structure and function of the methanogenic microbial communities in Uruguayan soils shifted between pasture and irrigated rice fields. 15, 2588–2602. doi:10.1111/1462-2920.12161.

Scheid, D., and Stubner, S. (2001). Structure and diversity of Gram-negative sulfate-reducing bacteria on rice roots. *FEMS Microbiol. Ecol.* 36, 175–183. doi:10.1016/S0168-6496(01)00131-3.

Scheid, D., Stubner, S., and Conrad, R. (2004). Identification of rice root associated nitrate, sulfate and ferric iron reducing bacteria during root decomposition. *FEMS Microbiol. Ecol.* 50, 101–110. doi:10.1016/j.femsec.2004.06.001.

Shrestha, M., Abraham, W. R., Shrestha, P. M., Noll, M., and Conrad, R. (2008). Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. *Environ. Microbiol.* 10, 400–412. doi:10.1111/j.1462-2920.2007.01462.x.

Shrestha, M., Shrestha, P. M., and Conrad, R. (2011). Bacterial and archaeal communities involved in the in situ degradation of ^{13}C-labelled straw in the rice rhizosphere. *Environ. Microbiol. Rep.* 3, 587–596. doi:10.1111/j.1758-2229.2011.00267.x.

Smalla, K., Wieland, G., Buchner, a, Zock, a, Parzy, J., Roskot, N., et al. (2001). Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis : Plant-Dependent Enrichment and Seasonal Shifts Revealed Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis. *Appl. Environ. Microbiol.* 67, 4742–4751. doi:10.1128/AEM.67.10.4742.

Snyder, C. S., Bruulsema, T. W., Jensen, T. L., and Fixen, P. E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agric. Ecosyst. Environ.* 133, 247–266. doi:10.1016/j.agee.2009.04.021.

Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R. S., Hess, W. M., et al. (1996). Taxol from *Pestalotiopsis microspora*, an endophytic fungus of Taxus wallachiana. *Microbiology* 142, 3–8. doi:10.1099/13500872-142-2-435.

Sugano, A., Tsuchimoto, H., Cho, T. C., Kimura, M., and Asakawa, S. (2005). Succession of methanogenic archaea in rice straw incorporated into a Japanese rice field: estimation by PCR-DGGE and sequence analyses. *Archaea* 1, 391–397. doi:10.1155/2005/582597.

Tardy, V., Chabbi, A., Charrier, X., De Berranger, C., Reignier, T., Dequiedt, S., et al. (2015). Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. *PLoS One* 10, 1–17. doi:10.1371/journal.pone.0130672.

Timsina, J., Jat, M. L., and Majumdar, K. (2010). Rice-maize systems of South Asia: Current status, future prospects and research priorities for nutrient management. *Plant Soil* 335, 65–82. doi:10.1007/s11104-010-0418-y.

Tuong, T. P., Bouman, B. a. M., and Mortimer, M. (2005). More rice, less water-integrated approaches for increasing water productivity in irrigated rice-based systems in asia. *Plant Prod. Sci.* 8, 231–241. doi:10.1626/pps.8.231.

Van Nguyen, N., and Ferrero, A. (2006). Meeting the challenges of global rice production. *Paddy Water Environ.* 4, 1–9. doi:10.1007/s10333-005-0031-5.

Wang, Z., Binder, M., Schoch, C. L., Johnston, P. R., Spatafora, J. W., and Hibbett, D. S. (2006). Evolution of helotialean fungi (*Leotiomycetes, Pezizomycotina*): A nuclear rDNA phylogeny. *Mol. Phylogenet. Evol.* 41, 295–312. doi:10.1016/j.ympev.2006.05.031.

Wassmann, R., Buendia, L. V, Lantin, R. S., Bueno, C. S., Lubigan, L. a, Umali, A., et al. (2000a). Mechanisms of crop management impact on methane emissions from rice fields in Los Banos, Philippines. *Nutr. Cycl. Agroecosystemsrient* 58, 107–119. doi:10.1007/978-94-010-0898-3_10.

Wassmann, R., Jagadish, S. V. K., Heuer, S., Ismail, A., Redona, E., Serraj, R., et al. (2009). *Chapter 2 Climate Change Affecting Rice Production. The Physiological and Agronomic Basis for Possible Adaptation Strategies*. 1st ed. Elsevier Inc. doi:10.1016/S0065-2113(08)00802-X.

Wassmann, R., Lantin, R. S., Neue, H. U., Buendia, L. V., Corton, T. M., and Lu, Y. (2000b). Characterization of methane emissions from rice fields in Asia. III. Mitigation options and future research needs. *Nutr. Cycl. Agroecosystems* 58, 23–36. doi:10.1023/A:1009874014903.

Weller, S., Janz, B., Jörg, L., Kraus, D., Racela, H. S. U., Wassmann, R., et al. (2016). Greenhouse gas emissions and global warming potential of traditional and diversified tropical rice rotation systems. *Glob. Chang. Biol.* 22, 432–448. doi:10.1111/gcb.13099.

Weller, S., Kraus, D., Ayag, K. R. P., Wassmann, R., Alberto, M. C. R., Butterbach-Bahl, K., et al. (2015). Methane and nitrous oxide emissions from rice and maize production in diversified rice cropping systems. *Nutr. Cycl. Agroecosystems* 101, 37–53. doi:10.1007/s10705-014-9658-1.

Wu, D., Zhao, Z., Han, X., Meng, F., Wu, W., Zhou, M., et al. (2018). Potential dual effect of nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrifier denitrification in the mitigation of peak N 2 O emission events in North China Plain cropping systems. *Soil Biol. Biochem.* 121, 147–153. doi:10.1016/j.soilbio.2018.03.010.

Xuan, D. T., Guong, V. T., Rosling, A., Alström, S., Chai, B., and Högberg, N. (2012). Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, Oryza sativa. *Biol. Fertil. Soils* 48, 217–225. doi:10.1007/s00374-011-0618-5.

Yagi, K., Tsuruta, H., Kanda, K.-I., and Minami, K. (1996). Effect of water managment on methane emission from a Japanese rice paddy field: Automated methane monitoring. *Global Biogeochem. Cycles* 10, 255–267. doi:10.1029/96gb00517.

Yang, C., and Crowley, D. E. (2000). Rhizosphere Microbial Community Structure in Relation to Root Location and Plant Iron Nutritional Status Rhizosphere Microbial Community Structure in Relation to Root Location and Plant Iron Nutritional Status. 66, 345–351. doi:10.1128/AEM.66.1.345-351.2000.Updated.

Yin, C., Jones, K. L., Peterson, D. E., Garrett, K. A., Hulbert, S. H., and Paulitz, T. C. (2010). Members of soil bacterial communities sensitive to tillage and crop rotation. *Soil Biol. Biochem.* 42, 2111–2118. doi:10.1016/j.soilbio.2010.08.006.

Yuan, Q., Pump, J., and Conrad, R. (2014). Straw application in paddy soil enhances methane production also from other carbon sources. *Biogeosciences* 11, 237–246. doi:10.5194/bg-11-237-2014.

Zhang, W., Li, Y., Xu, C., Li, Q., and Lin, W. (2016). Isotope signatures of N_2O emitted from vegetable soil: Ammonia oxidation drives N_2O production in NH^{4+} -fertilized soil of North China. *Sci. Rep.* 6, 1–10. doi:10.1038/srep29257.

Zhao, J., Zhang, R., Xue, C., Xun, W., Sun, L., Xu, Y., et al. (2014). Pyrosequencing reveals contrasting soil bacterial diversity and community structure of two main winter wheat cropping systems in china. *Microb. Ecol.* 67, 443–453. doi:10.1007/s00248-013-0322-0.

Zhao, M., Sun, B., Wu, L., Gao, Q., Wang, F., Wen, C., et al. (2016). Zonal soil type determines soil microbial responses to maize cropping and fertilization. *mSystems* 1, e00075-16. doi:10.1128/mSystems.00075-16.

5. Appendix

Eidesstattliche Erklärung

Ich versichere, dass ich meine Dissertation

„Introduction of crop rotation and rice straw application in a former flooded rice system and their impact on the microbial community in bulk soil and the rhizosphere of *Zea mays*“

selbständig und ohne unerlaubte Hilfe angefertigt habe und mich keiner als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe. Diese Dissertation wurde in der jetzigen oder ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

August 2018

Danksagung

An erster Stelle möchte ich mich ganz herzlich bei meiner Doktormutter, Frau Prof. Dr. Claudia Knief bedanken, die mir die Gelegenheit gegeben hat diese Doktorarbeit anzufertigen. Ich bedanke mich für alle Ratschläge, für die Freiheit mit der ich das Projekt bearbeiten durfte und dass ich wirklich mit allen Fragen und Problemen zu dir kommen könnte.

An nächster Stelle bedanke ich mich bei Herrn Prof. Dr. André Lipski für die Übernahme des Postens als Zweitgutachter für meine Doktorarbeit. Auch Herr Prof. Dr. Lukas Schreiber und Frau Elisabeth Soergel danke ich herzlich für die Übernahme des fachnahen und fachfremden Gutachters meiner Prüfungskommission.

Ein ganz besonderer Dank gilt Frau Dr. Katharina Frindte für alle guten Ratschläge, die super Zusammenarbeit und der gute Zuspruch. Über die Jahre der Zusammenarbeit bist Du, liebe Katharina von meiner Mentorin zur Freundin geworden. Ebenso wie Danh Biesgen, der mir nicht nur als Kollege sondern auch als Freund immer zur Seite stand. Danke, dass ihr eigentlich immer mehr an mich geglaubt habt als ich selbst und mich damit immer wieder neu motiviert habt. Ohne euch, eure aufmunternden Worte und euer Vertrauen in mich hätte ich es nicht geschafft.

Judith Harz, auch dir kann ich nicht genug für die Unterstützung im Labor danken. Ich hoffe es quält dich jetzt keiner mehr mit PLFA-Extraktionen. Wiebke Sickel und Marius Linnartz möchte ich für die Hilfe bei der Bioinformatik danken, ihr kamt genau zur richtigen Zeit. Romy Geer dir möchte ich danken, dass du die beste Masterstudentin warst die man sich vorstellen kann.

Die 6 Monate auf den Philippinen haben mir besonders Irabella Fuhrmann, Bernhard Liese und Janice Neumann versüßt. Aber auch den Feldarbeitern auf den Philippinen muss ich großen Dank aussprechen.

Nicht zu vergessen sind meine Kolleginnen Merle Noschinski, Tanja Schütte und Katharina Werner. Nicht nur für eure tatkräftige Unterstützung bei der Arbeit, sondern auch für eure Geduld und euer Verständnis danke ich euch sehr. Ihr seid über die Jahre schon fast wie eine zweite Familie geworden.

Aber auch allen anderen, die mich bei dieser Arbeit tatkräftig unterstützt haben, möchte ich natürlich danken. Dazu gehören die Bodenkunde (ganz besonders Gerd Welp, Kirsten Unger und Christian Hoffmann) sowie die Mitarbeiter in Klein-Altendorf. Danke, nicht nur für die Unterstützung sondern auch für die tolle Zusammenarbeit und gute Arbeitsatmosphäre.

Nicht zuletzt möchte ich meinen Schwestern Nadia Haxhaj und Sari Maarastawi sowie meinem Mann Thies Zoche danken. Ohne eure Liebe und Unterstützung wäre diese Arbeit nicht möglich gewesen.

Alle diese Menschen haben dazu beigetragen, dass meine Promotion eine interessante, lehrreiche und wunderbare Zeit war, danke nochmal an alle.