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Laufzeit des Vorhabens: **1.09.2014 - 31.08.2017**	
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DeltAdapt

HELMHOLTZ ZENTRUM FÜR UMWELTFORSCHUNG UFZ

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I. Kurze Darstellung / Short description

1. Aufgabenstellung / Tasks and objectives

The sub-project “Mitigation options using rice compost, fungi assisted” comprises research activities of the Department of Environmental Microbiology of the Helmholtz Centre for Environmental Research – UFZ (UFZ) in Germany and the Soil Biology Research Group of the Department of Soil Science of Can Tho University (CTU) in Vietnam, and has contributed to work package 3 (WP 3; Soil productivity and yield potentials) of the overall DeltAdapt project. In particular, it aimed to isolate and test lignocellulose-degrading fungi from rice straw and soils of the Mekong Delta (both salinity-affected and freshwater sites) and from the UFZ culture collection for their suitability to accelerate rice straw composting, and to select and transfer suitable fungi/consortia to Vietnam where sub-sequent pilot-scale composting and field application trials using compost were carried out. These project activities should help to clarify whether there is an option to mitigate soil degradation caused by topsoil removal and salinity intrusion in Vietnam’s Mekong Delta, using fungi assisted short-term composted rice straw.

2. Voraussetzungen, unter denen das Vorhaben durchgeführt wurde / Prerequisites for the conducted sub-project

The overall DeltAdapt project was coordinated by the Department of Soil Science (Prof. Dr. W. Amelung), Institute of Crop Science and Resource Conservation (INRES), University of Bonn. At UFZ, the Environmental Mycology Research Group (Dr. D. Schlosser and team) of the Department of Environmental Microbiology has long-term experience related to the physiology, biochemistry, enzymology, and biotechnology of the degradation of organic compounds by fungi of different eco-physiological groups and habitats, and the respective methodology. The UFZ team is further well experienced in international collaboration and was also involved in several EU-funded projects (SOPHIED, MINOTAURUS, BIOCLEAN). Within DeltAdapt, UFZ has contributed to composting studies of rice straw using fungal isolates and consortia in order to make this lignocellulosic compound more rapidly accessible for subsoil regeneration. The fungal strain collection of UMB, which contains about 250 fungal strains from various (partly highly saline) aquatic environments as well as various basidiomycetes covering different lignocellulose decay types, was made available for the project.

At CTU, the Soil Biology Research Group of the Department of Soil Science (Prof. Vo Thi Guong, Dr. Duong Minh Vien and team) is well experienced in soil biology, microbial ecology, and biochemistry. Substantial experience also exists with respect to international collaborations,

where the group has been involved in several third party-funded projects with other countries like e.g. Germany, Belgium, Netherland, Australia, USA, Sweden, Finland, Denmark, and others. The following examples illustrate projects focusing on composting and decomposition of rice straw and other agricultural wastes: (i) Making compost from water hyacinth as a bio-fertilizer to improve crop yields; (ii) Rice straw decomposition by fungi and bacteria; (iii) Biodiversity of microbial communities involved in decomposition of rice straw in different crop models in the Mekong Delta of Vietnam; and (iv) Isolation of plastic-degrading fungi from municipal waste landfills of the Mekong Delta of Vietnam.

3. Planung und Ablauf des Vorhabens / Planning and course of the project

The project-related activities were planned and implemented in close collaboration between UFZ and the Soil Biology Research Group of the Department of Soil Science, CTU. Work related to the tasks listed below carried out at UFZ was coordinated by UFZ, and work carried out in Vietnam was coordinated by CTU. The work was based on the experience, methodology and infrastructure available from UFZ and CTU. Methods and working procedures were appropriately adapted where needed. Work in the sub-project was aided by considering the pertinent international specialist literature, and organized according to the following major tasks:

Work carried out at UFZ

- Isolation of fungi from rice straw and soil samples of the Mekong delta
- Lab-scale evaluation of rice straw decomposition abilities of isolates/consortia under sterile and non-sterile conditions, selection of the most promising isolates/consortia for composting trials and transfer of the most suitable strains/consortia to Vietnam

Work carried out in Vietnam / CTU

- Initial site survey, selection of sampling sites and sampling in the Mekong delta
- Pilot-scale composting trials and preceding production of fungal inocula
- Field trials addressing effects of produced compost on soil properties and plant performance

4. Wissenschaftlicher und technischer Stand, an den angeknüpft wurde / Scientific and technical state serving as a starting point

4.1. Wissenschaftlich-technische Ausgangspunkte / Scientific and technical starting points

Incorporation of composts, based on local agricultural by-products might be one promising option for local shareholders to compensate losses of soil organic matter (SOM) and nutrients associated with soil removal to recover soil fertility and crop yield. Rice straw accounts for approximately 50% of the total rice biomass (Putun and Apaydin, 2004), and is mainly composed of cellulose and hemicelluloses (~74%), lignin (~18%), fat (~1%), and protein (~3%) (Phuong et al., 2010). The annual rice production volume in the Mekong Delta region is more than 18.7 million tons, hence generating about 4.5 million tons of rice straw per year. While some of this waste is used for food, animal feeding, or recycled into straw bricks, approximately 70% is buried or burned, thereby generating considerable GHG emissions (The World Bank Group, 2012). Only a very small amount of the total rice straw in the Mekong and Red River Deltas is used to produce bio-fertilizers by composting. Nevertheless, composting of rice straw and subsequent application as bio-fertilizer to salt-affected soils represents a very promising approach contributing to sustainable adaptation of agro-ecosystems in the Mekong Delta to increased salinity intrusion.

Traditional rice straw composting (i.e. anaerobic or aerobic decomposition, the latter one using passive aeration) usually takes 3 to 4 months until compost maturity is achieved. It requires a comparatively big composting area, and represents the least expensive composting method without extensive labor inputs. However, this composting method is to slow when two or even more crops per year have to be considered (Afrizal, 2009), which applies to the Mekong and Red River Delta. Thus, accelerating the composting process is crucial for the intended use of compost in the Deltas. Rapid composting methods based on the amendment with lignocellulose-decomposing microbes to accelerate aerobic decomposition have already been developed. The so-called IBS rapid composting method (IBS = Institute of Biological Sciences, Philippines) uses a preparation of the cellulolytic fungus *Trichoderma harzinum* as a fungal activator, reducing composting time to only 3 to 4 weeks (Cuevas, 1993). Besides a carbon-rich material such as rice straw, the process also requires nitrogen-rich materials like animal manure and nitrogen-rich plant wastes. Such additives may not always be available at sufficient amounts, hence resulting in compost with low nitrogen content. Further drawbacks of this composting method are that (i) it is more laborious than conventional composting, (ii) it depends on a continuous supply with the fungal activator *Trichoderma harzinum* which cannot be

guaranteed everywhere and may be critical especially under small farms conditions, and (iii) microbial contaminants / other microbes present in the substrate(s) may reduce the effectiveness of the fungal activator (Afrizal, 2009; Cuevas, 1993). The so-called bio-enriched methods also rely on the action of a fungal activator, and additionally employ other nitrogen-fixing microbes intended to enhance the nitrogen content of mature compost. For this, nitrogen-fixing bacteria of the genus *Azotobacter* are applied after an initial composting period using *T. harzinum* for 2 to 3 weeks, and composting is continued for another week until an N-enriched compost is achieved (Afrizal, 2009). This partly solves the problem with low nitrogen concentration but possible drawbacks of the bio-enriched approach are the same as for the IBS rapid composting method.

Fungi are primary decomposers of complex lignocellulose-based plant materials, with lignin representing the most recalcitrant plant cell wall constituent being efficiently degraded only by certain fungi (Baldrian, 2008; Huang et al., 2008). Lignin is known to hamper the accessibility of plant cellulose and hemicelluloses to microbial hydrolysis and further microbial utilization, and its removal and bioconversion into humic substance-like compounds is thought to be a major process influencing compost maturation (Huang et al., 2008). Fungi acting on both lignin and polysaccharide constituents of plant cell walls are therefore preferential candidates of choice for accelerating rice straw composting processes. Besides essentially polysaccharide-hydrolyzing *Trichoderma* species which have widely been considered for composting purposes because of their strong cellulolytic (and in the case of the biocontrol fungus *T. harzinum* also chitinolytic) activities, lignin-degrading fungi of the white-rot decay type of lignocellulose have also been investigated and demonstrated to promote delignification and humification of rice straw (Do Vale et al., 2012; Huang et al., 2008; Yu et al., 2009).

The successful application of non-autochthonous fungi for composting purposes requires their sufficiently stable establishment and survival in the non-sterile rice straw (or husk) compost environment. Besides suitability for the particular substrate, compatibility with other accompanying environmental conditions and the native micro-flora or (in case of competition / antagonistic interactions with native microbes), competitive abilities / the ability to suppress antagonists would be a prerequisite. Concerning the application of ligninolytic fungi to non-native environments, related effects and requirements have extensively been investigated and discussed in the context of soil bioremediation (Baldrian, 2008). Cellulolytic fungi such as *T. harzinum* at the same time are strong biocontrol agents (Do Vale et al., 2012), thus facilitating survival in non-native and non-sterile environments. Nevertheless, under the potentially competitive/antagonistic conditions of a complex native micro-flora already present, as well as

under otherwise adverse environmental conditions the application of fungal consortia could be more promising than relying on single species. For instance, it was demonstrated that a fungal consortium composed of three species representing cellulolytic as well as ligninolytic fungi caused a more efficient saccharification of wheat straw than only *Trichoderma* sp., which was used as a control (Lin et al., 2011). Fungal consortia were also successfully applied for composting of rice straw (Kausar et al., 2010). Current research results of partner UFZ indicate that increasing the fungal diversity (i.e. increasing the number of fungal species) of constructed fungal consortia may enhance the robustness to degrade environmental pollutants under stress conditions. Similar beneficial effects of an increased biodiversity were already reported for bacteria-protist interactions (Saleem et al., 2013).

The specific enrichment and selection of autochthonous fungi from the sites and materials intended for their use, and their application for composting in an enriched form represents another possibility to avoid or at least mitigate problems related to the compatibility with the substrate / compost environment. This approach could be of particular interest with respect to adaptation to saline conditions. The utility of the application of such fungi, which are adapted to saline habitats, has successfully been demonstrated for the biological treatment of highly saline/alkaline effluents of the dyeing industries (D'Souza et al., 2006; Raghukumar et al., 2008).

4.2. Methodische Vorgehensweise / Methodological approach

The following methodological approach was deduced from the scientific and technical state explained in sub-section 4.1. above. An initial review of relevant international and local literature with respect to the different use options of rice straw and husk re-use in the delta, and a comprehensive survey targeting the present use of rice straw and husk after harvesting aimed to gather information about the regional distribution of raw rice straw and husk materials and how this is affected by the increased salinity intrusion. The related information was used to support a decision on the selection of suitable sampling sites (a representative site in the freshwater area and one influenced by salinity) for the isolation of autochthonous lignocellulose-decomposing fungi.

Site-adapted autochthonous fungi, which are capable of decomposing rice straw, were isolated from soil and rice straw at UFZ in Germany after transfer of samples from Vietnam. Samples were taken at one saline (rice-shrimp system) and one freshwater (rice system) site of the Mekong delta (all in Thanh Phu district, Ben Tre province). Agitated enrichment cultures containing suspended rice straw as the only growth substrate were consecutively transferred into new media in short intervals, to enrich fast-growing fungi (bacteria were suppressed using

appropriate mixtures of antibiotics). Serial dilutions from such enrichments were plated out on agar plates containing rice straw as the only growth substrate, and pure cultures were obtained by picking single colonies. In addition, extracts from samples were directly used for fungal isolation without enrichment. Cultivations were carried out under conditions mimicking those of the sampling sites with respect to temperature, pH, and salinity.

The newly obtained isolates were assessed regarding their efficiency to decompose rice straw under sterile as well as non-sterile conditions. In addition, the performance of fungal consortia constructed from the obtained isolates was also assessed. As an exit strategy addressing the possibility that sufficiently efficient and robust autochthonous fungal isolates could not be obtained, a range of promising fungal strains taken from the fungal strain collection of UFZ (fungi from various partly highly saline aquatic environments as well as various wood-rotting basidiomycetes; which were chosen on the basis of known characteristics/already available data) was also tested. Solid-state cultivations were carried using small amounts (0.5 g) of rice straw under conditions mimicking those of the sampling sites with respect to temperature, pH, and salinity. Sterilized as well as non-sterile rice straw substrates without fungal inoculation served as controls. Parameters like total dry mass, lignin content (determination via Fourier-transform infrared spectroscopy = FTIR spectroscopy), and fungal biomass (using ergosterol as a biomass marker) were determined and used to calculate mass balances and decomposition rates, in order to enable a quantitative evaluation of the efficiency of decomposition. The activity of lignin-modifying enzymes was concomitantly monitored.

The taxonomic identification of the most promising ones among the newly isolated strains was aided by the Belgian Coordinated Collections of Microorganisms/Mycothèque de L'Université catholique de Louvain (BCCM/MUCL, Louvain-la-Neuve, Belgium). Based on the obtained results, the most efficient strains/consortia were selected for application in pilot-scale compost trials in Vietnam, and transferred to Vietnam for this purpose.

Pilot-scale rice-straw composting trials were conducted at the Department of Soil Science, CTU, Vietnam. Fungal inoculum production in sterile plastic bags using cooked (sterile) rice as a substrate was carried out prior to fungal inoculation of the piles. After that, the produced fungal inocula were applied to compost piles (rice straw volume about 1 m3). Pilot-scale composting processes were followed over a total period of 68 days.

Composted rice straw was applied in subsequent field trials, which addressed the possible amelioration of soil fertility in topsoil-removed rice paddy fields by amending organic matter. Related experiments were carried out in Soc Trang province (My Xuyen district) and Tra Vinh province (Chau Thanh district) (involved DeltAdapt partners: CTU/Vietnam; INRES/Germany).

Rice straw-based compost derived from composting with fungi was also applied to soil of citrus orchards in Vinh Long province, in order to study possible effects on (i) the abundance of soil-borne plant-pathogenic *Fusarium* sp. strains and (ii) a possible reduction of the thereof resulting dry root rot disease of plants in citrus orchards (involved Vietnamese partners: Department of Soil Science, CTU).

5. Zusammenarbeit mit anderen Stellen / Cooperation with others

In addition to a particularly close collaboration with CTU's Department of Soil Science, intensive information and data exchange was realized with all DeltAdapt partners within the frame of the project meetings and bilateral contacts. Especially the coordinator INRES and the Institute for Environment and Human Security, United Nations University (UNU) provided valuable support with respect to the organization and coordination of research activities. Furthermore, the responsible authority of the Free State of Saxony (*German* Referat Pflanzengesundheit, Sächsisches Landesamt für Umwelt, Landwirtschaft und Geologie) was involved in order to obtain a permission to import rice straw and soil samples from Vietnam.

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II. Eingehende Darstellung / Detailled description

1. Verwendung der Zuwendung und des erzielten Ergebnisses im Einzelnen, mit Gegenüberstellung der vorgegebenen Ziele / Use of the grant and the achieved outcome in comparison with the targeted objectives

Overall, the UFZ sub-project “Mitigation options using rice compost, fungi assisted” was part of WP 3 and aimed to isolate and test lignocellulose-degrading fungi from rice straw and soils of the Mekong Delta (both salinity-affected and freshwater sites) and from the UFZ culture collection for their suitability to accelerate rice straw composting, and to select and transfer suitable fungi/consortia to Vietnam where sub-sequent pilot-scale composting and field application trials using compost were intended to be carried out. These project-related activities should help to clarify whether there is an option to mitigate soil degradation caused by topsoil removal and salinity intrusion in Vietnam’s Mekong Delta, using fungi assisted short-term composted rice straw. Work carried out at UFZ and in Vietnam was organized according to the tasks and objectives shown in Table 1, in order to reach the project goals. The allocated resources were used to support the work carried out by UFZ accordingly.

Table 1: Major tasks and objectives of the UFZ sub-project “Mitigation options using rice compost, fungi assisted”, and distribution of related work between UFZ and the Vietnamese project partner CTU (Department of Soil Science).

Tasks	**Objectives**
Work carried out at UFZ	
Isolation of fungi from rice straw and soil samples of the Mekong delta	Promising strains available for detailed evaluation of their decomposition abilities
Lab-scale evaluation of rice straw decomposition abilities of isolates/consortia under sterile and non-sterile conditions, selection of the most promising isolates/consortia for composting trials and transfer of the most suitable strains/consortia to Vietnam	Promising strains/consortia for inoculation of pilot-scale composting trials are selected and available in Vietnam
Work carried out in Vietnam / CTU	
Initial site survey, selection of sampling sites and sampling in the Mekong delta	Samples for enrichment and isolation of fungi taken and available at UFZ
Pilot-scale composting trials and preceding production of fungal inocula	Efficiency of fungi/consortia for composting evaluated at pilot scale
Field trials addressing effects of produced compost on soil properties and plant performance	Compost effects on soil properties and plant performance assessed

1.1. Initial site survey, selection of sampling sites and sampling in the Mekong delta

During the DeltAdapt kickoff workshop and accompanying field trips in the Mekong delta, which had been organized by the Vietnamese partners, several potential sampling sites were surveyed by the Vietnamese (CTU) and German partners (UFZ). Details for sampling and sample processing were fixed. Also considering the results of a related literature review conducted at CTU, one saline (rice-shrimp system) and one freshwater (rice system) site of the Mekong delta (all from Thanh Phu district, Ben Tre province) were sampled by the Vietnamese partners, with the aim to isolate autochthonous fungi from them later on at UFZ. Rice straw and soil samples from each site were transferred to UFZ by a Vietnamese colleague (matching PhD student) involved in the project.

1.2. Isolation of fungi from rice straw and soil samples of the Mekong delta

Autochthonous fungi from the rice straw and soil sites were isolated at UFZ. In total, 34 filamentous fungal strains were obtained from rice straw and soil from the freshwater site, and 29 filamentous fungi and two yeast-like fungi were obtained from rice straw and soil from the saline site (**Fig. 1**, **Table 2**). The obtained isolates have been quantitatively tested for growth on rice straw on agar plates. Furthermore, they were tested for the formation of lignin-modifying oxidases and peroxidases, and for the formation of cellulases on agar media.

Five strains from the saline and 5 strains from the freshwater site growing most rapidly on rice straw, respectively, were selected for further experiments intended to address their efficiency in the decomposition of rice straw under sterile and non-sterile solid-state conditions. Five additional fungal strains chosen from the UFZ strain collection were additionally foreseen for these experiments, in order to maximize the chances for a successful selection of candidate strains to be used in the pilot-scale composting trials in Vietnam.

Table 2: Numbers of fungal isolates obtained from samples of the different sampling sites in Vietnam's Mekong delta (Thanh Phu district, Ben Tre province).

Sample type	**No. of obtained fungal isolates**
Freshwater site / soil	10
Freshwater site / rice straw	24
Saline site / soil	19
Saline site / rice straw	12

Fig. 1: Examples for fungal isolates on agar plates, which were obtained from samples of the different sampling sites in Vietnam's Mekong delta (two strains from the freshwater and the saline site, respectively).

1.3. Lab-scale evaluation of rice straw decomposition abilities of isolates/consortia under sterile and non-sterile conditions, selection of the most promising isolates/consortia for composting trials, and transfer of the most suitable strains/consortia to Vietnam

Based on the fungal isolates obtained as reported above, 5 promising strains (i.e. those growing most rapidly on agar plates containing rice straw as the sole source of carbon and energy) from the saline sampling site (rice-shrimp system) and 5 likewise selected promising strains from the freshwater sampling site (rice system; all sites located in Thanh Phu district, Ben Tre province, Mekong delta) were applied in laboratory-scale experiments, in order to assess the fungal efficiency in decomposing rice straw under sterile and non-sterile solid-state cultivation conditions (applied amount of rice straw: 0.5 g, respectively; C:N ratio of about 34:1). Experiments were carried out under conditions mimicking salinity, pH and temperature of the respective sampling site. In addition to the Vietnamese strains mentioned before, 5 promising fungal strains chosen from the UFZ strain collection were also investigated under both freshwater and saline conditions; in accordance with the original research plan. Total dry mass loss, lignin loss (by means of FTIR spectroscopy), and fungal biomass (by means of ergosterol analysis) were determined and used to evaluate the efficiency of decomposition.

Based on the obtained results, a second set of experiments was conducted in order to compare the efficiency of constructed fungal consortia in rice straw decomposition with that of the most efficient single isolate obtained from either Vietnamese sampling site (i.e. saline and freshwater). Consortia were constructed from the 5 strains performing most efficiently under non-sterile conditions, respectively. Likewise, the single strains used for comparison were selected among the Vietnamese strains performing most efficiently under non-sterile conditions,

respectively. Concomitantly, the size of the respective fungal inoculum used to inoculate the solid-state cultures was increased in order to improve survival under non-sterile conditions. The C:N ratio was always adjusted to about 28:1. Again, experiments were carried out under sterile and non-sterile conditions, and under conditions mimicking salinity, pH and temperature of the respective sampling site.

Results are exemplified in **Fig. 2** and suggest that under certain circumstances it was possible to accelerate the decomposition of rice straw components by fungal inoculation even under non-sterile conditions.

The most promising ones among the newly isolated Vietnamese strains were sent to the Belgian Coordinated Collections of Microorganisms/Mycothèque de L'Université catholique de Louvain (BCCM/MUCL, Louvain-la-Neuve, Belgium) for taxonomic identification. The identification results were made available to the Vietnamese partners. Based on the results of the aforementioned experiments and the fungal identification, a list of strains to be transferred to Vietnam for use in the planned pilot-scale composting trials in Vietnam was established. This included the 8 most promising and taxonomically identified strains from the Vietnamese sampling sites shown in **Table 3**, 3 successfully tested and promising strains with known identity from the UFZ collection, and 8 non-identified Vietnamese strains for comparison. These strains were transferred to Vietnam for further experiments, in accordance with the initial planning.

Table 3: Results of the taxonomic identification of the 8 most promising fungal strains isolated from sampling sites in Vietnam's Mekong delta. Two further identified strains not listed in the table turned out to be plant-pathogenic species, and were excluded from further experiments.

Strain designation	Taxonomic information
FR 2-1	*Nigrospora* sp.
FS 4-3	*Rhizomucor variabilis* R.Y. Zheng & G.Q. Chen
FS 3-2	*Trichoderma asperellum*
SR 8-3	*Phoma herbarum* Westendorp
SR 8-1	*Nigrospora* sp.
SS10^{o} D1	*Trichoderma asperellum*
SR 5-1	*Phoma herbarum* Westendorp
SS10^{-1} E1	*Gongronella butleri* (Lendner) Peyronel & Dal Vesco

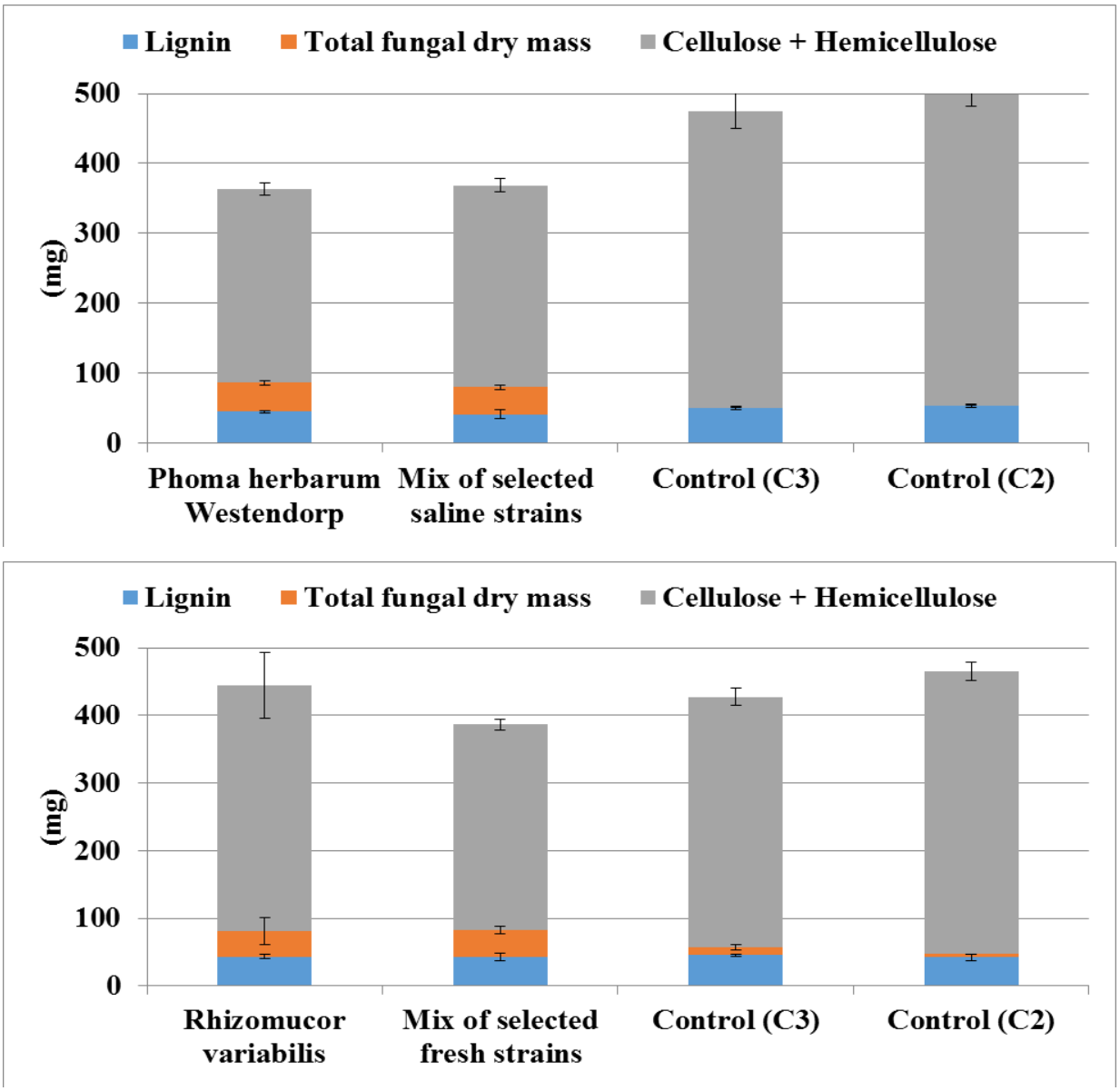

Fig. 2: Examples for fungal treatment of wheat straw (2 weeks at 28 °C) under sterile saline (upper figure) and non-sterile freshwater conditions (lower figure) at laboratory scale. For further information about single strains and constructed consortia (mix of selected strains) please refer to the text. Mass balances (dry mass basis) were generated upon analysis of total dry mass, fungal biomass (based on ergosterol content), and lignin content (using FTIR spectroscopy). Cellulose + hemicellulose contents were calculated as the difference between total dry mass and the sum of fungal biomass and lignin content. C2 and C3 represent uninoculated controls at the beginning (C2) and the end of the experiment (C3). Data represent means ± standard deviations from triplicate experiments.

1.4. Pilot-scale composting trials and preceding production of fungal inocula

Following an extensive exchange of ideas and knowledge with the Vietnamese partners (Department of Soil Science, CTU), strains/fungal consortia were decided to be employed in pilot-scale rice-straw composting trials conducted at CTU as follows:

The taxonomically identified Vietnamese strains FS 4-3 (from the freshwater site) and SR 8-3 (from the saline site) were used as single strains to inoculate composting piles (about 1 m^3 rice straw volume), respectively (**Fig. 3**). A consortium composed of 6 out of the 8 identified Vietnamese strains (compare **Table 3**; the 2 *Trichoderma* strains were omitted to ensure comparability with additional composting trials, see below) was also applied in an additional pile (denoted as "Mix of all strains" in **Fig. 4** below). Another composting pile was inoculated with a commercial *Trichoderma* strain preparation, which is available from CTU, for comparison. An uninoculated composting pile served as control.

Fungal inoculum production in sterile plastic bags using cooked (sterile) rice as a substrate was carried out prior to fungal inoculation of the piles. After that, the produced fungal inocula were applied to the related compost piles and the pilot-scale composting experiments were started. **Fig. 3** illustrates the general experimental set-up used for the composting trials performed at CTU.

Fig. 3: Schematic illustration of the general experimental set-up used for the pilot-scale composting trials performed at the Department of Soil Science, CTU.

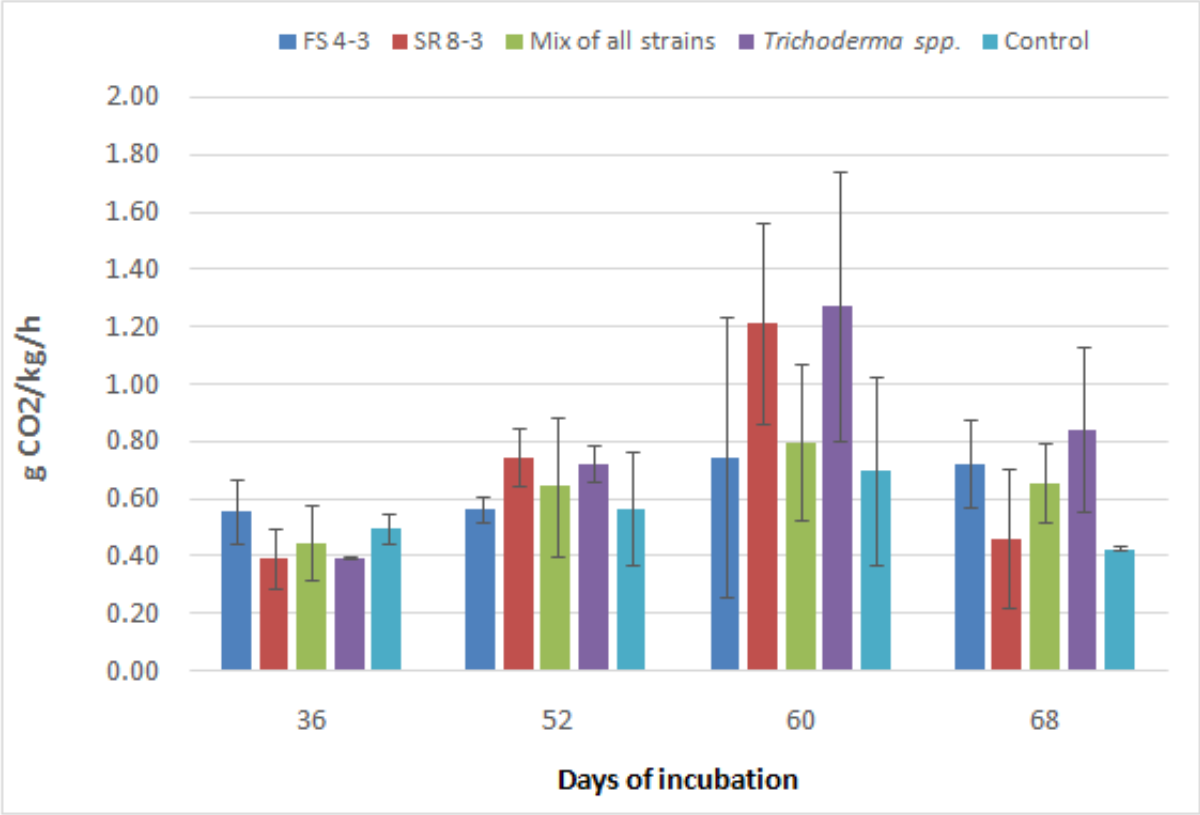

Fig. 4. CO_2 emission from pilot-scale rice straw composting piles operated at CTU. Please refer to the text for information about inoculation of the respective piles. Remarkably, the isolated strain SR 8-3 (*Phoma herbarum*) performed equally efficient compared to a commercial *Trichoderma* strain (available from CTU) during a composting period of 60 days. Also, the fungal consortium constructed from the isolated strains ("Mix of strains") was more efficient than the control after 68 days of incubation.

The composting trials were successfully accomplished within a period of 68 days, and previously isolated indigenous fungal strains could be demonstrated to be applicable for composting purposes (**Fig. 4**). Remarkably, the isolated strain SR 8-3 (*Phoma herbarum*) performed equally efficient compared to a commercial *Trichoderma* strain (available from CTU) during a composting period of 60 days. Furthermore, the fungal consortium constructed from the isolated strains ("Mix of strains" in **Fig. 4**) was more efficient than the control at a composting period of 68 days, respectively.

1.5. Field trials addressing effects of produced compost on soil properties and plant performance

Application of compost to rice fields

Fungi-assisted composted rice straw was made available for subsequent field trials, which addressed the possible amelioration of soil fertility in topsoil-removed rice paddy fields by amending organic matter. Related experiments were carried out in Soc Trang province (My Xuyen district) and Tra Vinh province (Chau Thanh district). The applied treatments involved the application of inorganic fertilizer (NPK) at two doses (i.e. current farmers' practice: 100 N - 60 P_2O_5 - 50 K_2O kg/ha; and the recommended application: 100 N - 30 P_2O_5 - 30 K_2O kg/ha), DeltAdapt (rice straw-based) compost at two doses (6 and 12 tons/ha; inorganic NPK fertilizer was always concomitantly applied at its recommended dose as above), and 10 tons/ha conventional compost from sugarcane-filter cake (a biowaste of sugar-processing factories) together with inorganic NPK fertilizer at its recommended dose as above (Vietnamese partners involved: Prof. Vo Thi Guong, Dr. Duong Minh Vien, Dr. Chau Minh Khoi, Tran Huynh Khanh, Duong Van Nam, CTU; German partners involved: Prof. Wulf Amelung; D.r Jens Kruse; Susanne Weigand, INRES).

Amendment with all types of compost generally significantly increased the organic C content in the subsoils (**Fig. 5**). Different from conventional compost from sugarcane-filter cake (which was applied during 4 consecutive crops in Soc Trang province), rice straw-based compost produced using fungi did not increase soil available N and P and the rice yield when applied during the last two crops at Soc Trang province. Remarkably, the fungi-derived rice straw compost did not decrease rice yields although the dose of the simultaneously applied inorganic NPK fertilizer was less than the NPK fertilizer dose currently applied by farmers. Thus, it can be concluded that a continuous long-term amendment with fungi inoculated rice straw-based compost offers a potential for decreased mineral fertilizer inputs, increased rice yields and/or improved soil quality of topsoil-removed rice paddy fields.

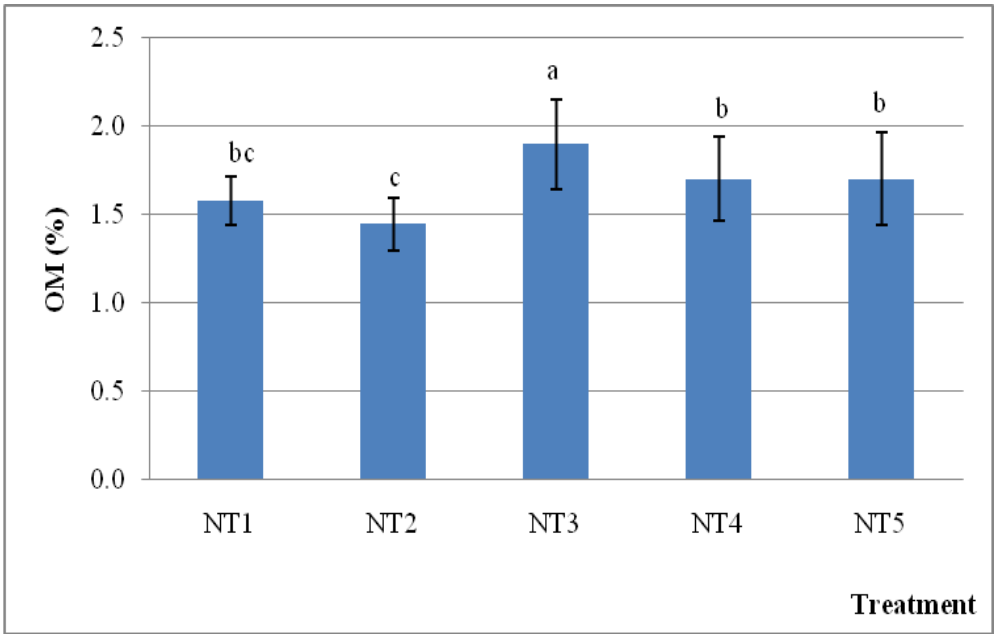

Fig. 5: Organic C content (organic matter; OM in %) of soil after the fourth crop in Soc Trang province. Treatment NT1: inorganic NPK fertilizer according to the current farmers' practice; NT2: inorganic NPK fertilizer at the recommended dose; NT3: conventional compost application; NT4: DeltAdapt (rice straw-based) compost applied at 6 tons/ha; NT5: DeltAdapt compost applied at 12 tons/ha. Please refer to the text for more experimental details.

Application of compost to soil of citrus orchards

Rice straw-based compost derived from composting with fungi (referred to as bio-fertilizer = BF later on) was also applied to soil of citrus orchards in Vinh Long province, in order to study possible effects on (i) the abundance of soil-borne plant-pathogenic *Fusarium* sp. strains and (ii) a possible reduction of the thereof resulting dry root rot disease of plants in citrus orchards (involved DeltAdapt partners: CTU/Vietnam; INRES/Germany). Related field experiments involved two groups of citrus plants, a dry root rot-diseased plant group and a healthy citrus plant group (48 plants/group). Soil treatments were carried out as follows: T1 - inorganic fertilizer (farmers' practice), T2 - inorganic fertilizer (recommended practice), T3: bio-fertilizer (BF) based on the known bio-control agent *Trichoderma asperellum* (isolated from citrus orchards), T4: BF based on *Gongronella butleri* (isolated at UFZ from Vietnamese samples and subsequently transferred to Vietnam), T5: BF based on *Trichoderma asperellum* + *Gongronella butleri*, and T6 - a commercial *Trichoderma* sp. product.

Notably, BF based on isolated indigenous fungi decreased the cell densities of potentially plant-pathogenic *Fusarium* sp. by about 42 to 58%, and dry root rot disease (in terms of yellow = diseased leaves) by 54 to 98% (**Fig. 6**). The combination of *Trichoderma asperellum* and *Gongronella butleri* reduced *Fusarium* sp. cell densities by 42% and dry root rot disease by 54%. The highest reduction in *Fusarium* sp. cell counts (by 58%) and dry root rot disease of citrus (by 98%) was observed with the *Gongronella butleri*-based BF. These tremendous effects of the rice straw-based compost on the suppression of the economically important dry root rot disease of citrus suggests that the indigenous fungi applied for composting may represent very efficient bio-control agents.

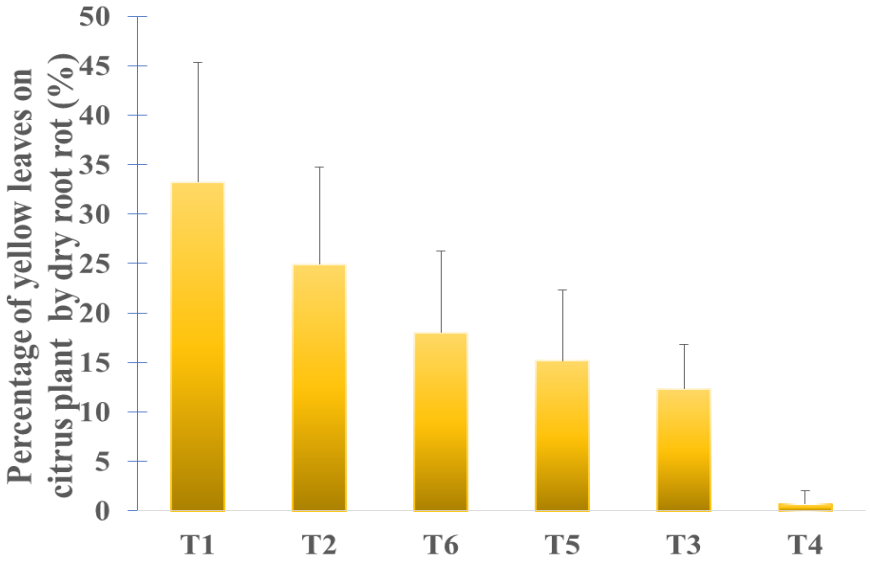

Fig. 6: Effects of amendments of soil of citrus orchards on the extent of dry root rot disease of citrus plants (based on counts of yellow = diseased leaves). T1 - inorganic fertilizer (farmers' practice), T2 - inorganic fertilizer (recommended practice), T3: bio-fertilizer (BF) based on the known bio-control agent *Trichoderma asperellum* (isolated from citrus orchards), T4: BF based on *Gongronella butleri* (isolated at UFZ from Vietnamese samples and subsequently transferred to Vietnam), T5: BF based on *Trichoderma asperellum* + *Gongronella butleri*, and T6 – a commercial *Trichoderma* sp. product.

2. Zahlenmäßiger Nachweis / Record of expenditure

A separate record of all project costs was provided by the UFZ Department of Scientific Administrative Project Supervision (WAP). Major positions include personnel costs of a researcher in charge of the work at UFZ (about 13,369 €), costs for traveling (about 5,708 €), administration costs (about 2,363 €) and materials (about 1,248 €).

3. Notwendigkeit und Angemessenheit der geleisteten Arbeit / Necessity and appropriateness of the work undertaken

The planning and execution of work was strictly based on the project tasks and objectives as outlined in the project application. It would not have been possible to carry out the present project without the granted funding, which ensured a successful accomplishment of the planned work within the foreseen time frame even against the background of challenging logistics and coordination requirements (i.e. distribution and coordination of work carried out in Germany and Vietnam, including the related logistics requirements). Additional resources were not requested.

4. Voraussichtlicher Nutzen und Verwertbarkeit der Ergebnisse / Presumable use and applicability of project results

Within the current project, indigenous fungi from could be obtained from rice straw and soil samples derived from a freshwater- and a salinity-affected site in the Mekong delta of Vietnam. Their potential to support composting of rice straw could be demonstrated at laboratory and pilot scale. Field application trials (soil amendment) with fungi-assisted rice straw compost suggest a potential for decreased mineral fertilizer inputs, increased rice yields and/or improved soil quality through continuous compost application over longer time periods. Furthermore, indigenous fungi applied for composting may represent very efficient bio-control agents. This conclusion could be drawn from a tremendous suppression of the economically important dry root rot disease of citrus and related microbial causes upon application of rice straw-based compost, which was demonstrated within the project. These scientifically very valuable results are intended to be published in ISI-listed scientific journals (see sub-section 6.1. below).

Furthermore, results of the project made an important contribution to a current PhD project (see sub-section 6.4. below). They were also integrated into the training programmes of 3 MSc students, 6 undergraduate students, and the related final theses. In addition, the project results will be used for teaching purposes (lectures dealing with soil fertility in the frame of soil science and agriculture science courses at CTU).

At the same time, the project results provide an excellent basis for a continuation and intensification of the scientific collaboration between Germany and Vietnam. In Vietnam, studies aiming at the identification and use of effective fungi (such as *Gongronella* sp. or *Trichoderma* sp.) to control soil-borne diseases in fruit orchards and upland crops of the Mekong delta are intended to be continued. Options to mitigate soil degradation through compost application particularly in salinity-affected areas are of great interest. Potential subjects of further scientific-technological collaboration therefore centre on the in-depth exploitation of site-adapted

indigenous micro-organisms for the production of biowaste-based fertilizers, which simultaneously possess bio-control agent properties. Related activities should also include a sound verification of the concept applicability at field scale. In case of success, such activities would be expected to facilitate the implementation of related measures into local farmers' practice; thereby also holding promise for a possible combination of potential economic and ecological benefits.

5. Während der Durchführung des Vorhabens bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen / Progress in the relevant subject area made by others during the course of the project

To date, we are not aware of progress made by others going beyond the results achieved in regard to the specific approach of the current project (i.e. beneficial effects through the use of indigenous, site-adapted fungal inocula; especially with respect to the potential use for the production of bio-fertilizers with bio-control agent properties).

Until now, certain kinds of straw are considered to be difficult to compost (Zhang et al., 2016). Several strategies are currently followed to improve straw composting. These are often based on co-composting of straw and more nitrogen-rich substrates, sometimes in combination with the use of rather "traditional" fungal inocula such as spent mushroom substrate, other known lignocellulose degraders, or *Trichoderma* sp. (Meng et al., 2018; Song et al., 2014; Zhou et al., 2015).

Recent studies also consider the potential impact of soil amendment with compost on the biodegradation of environmental pollutants, e.g. pesticides (Ren et al., 2018).

6. Erfolgte oder geplante Veröffentlichungen der Ergebnisse / Realized or planned publication of project results

6.1. Planned publications in ISI-listed journals

One publication dealing with the isolation of indigenous fungi from rice straw and soil samples of the Mekong delta, and the evaluation of their rice straw decomposition abilities in the light of a possible use for the acceleration of rice straw composting is currently planned.

One publication dealing with effects of rice straw composted with the help of indigenous fungal isolates on citrus plants and soil-borne plant-pathogenic *Fusarium* sp. in citrus orchards is currently planned.

6.2. Other publications

A manuscript (in Vietnamese) was submitted to the scientific journal of CTU: Some selected physical-chemical and biological soil properties related to dry root rot disease on *Citrus nobilis* in Tam Binh district, Vinh Long province.

6.3. Oral presentations at workshops and project meetings

Chau Minh Khoi (2017) Amelioration of soil fertility in topsoil-removed rice paddy fields by amending organic matter. DelpAdapt public workshop, 28 - 29 June 2017, Hanoi City, Vietnam.

Dietmar Schlosser (2017) Use of fungi for the acceleration of rice straw composting. DelpAdapt public workshop, 28 - 29 June 2017, Hanoi City, Vietnam.

Nguyen Ngoc Thanh, Duong Minh Vien, Dietmar Schlosser (2016) Towards acceleration of rice straw composting using fungi. DeltAdapt Midterm Meeting, 7 – 9 June 2016, Bonn, Germany.

Nguyen Ngoc Thanh (2017) Effect of inoculation of isolated fungi strains on improvement of some selected soil properties of citrus orchards. DelpAdapt public workshop, 28 - 29 June 2017, Hanoi City, Vietnam.

6.4. PhD thesis

The results of the present project represent an important part of the PhD thesis of matching PhD Nguyen Ngoc Thanh, which is currently in preparation and intended to be submitted in 2018.

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