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Nitrogen sequestration in paddy and non-paddy soils formed from different parent materials

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SUMMARY

Nitrogen sequestration in paddy and non-paddy soils formed from different parent materials

Paddy soils exhibit low nitrogen (N) use efficiency. Prior research in this field suggested that paddy soils may sequester significant amounts of N in aged amino acids, microbial residues, or even in pyrogenic N. The overarching aim of my theses was to identify these bonding forms of N sequestration in different soil types, and to elucidate to what extent the properties of the parent soil rather than anthropogenic soil management controls the different N sequestration processes.

My hypothesis was that the effects of rice cultivation on N sequestration in soil depend on the mineral composition of the parent soil. My specific research questions were: (i) does paddy management lead to N sequestration in peptide bonds, (ii) do bacteria or fungi promote high microbial N sequestration in paddy soils, and (iii) to which extent can N sequestration in paddy soils be assigned to the input of charred organic matter?

To answer these research questions, I sampled paddy and adjacent non-paddy top- and subsoils from different major reference soil groups (Vertisols, Andosols, Alisols, and a Gleysol/Fluvisol pair), allocated in major paddy rice production regions in Indonesia, China and Italy. I used N biomarkers like amino sugars and amino acid enantiomers to elucidate whether N sequestration in peptides and microbial residues is stronger under paddy compared with non-paddy management. Selected samples were pre-extracted with dithionite–citrate–bicarbonate (DCB) to better understand the role of reactive pedogenic oxides to amino acid-N storage, origin and composition. Additionally, I analyzed charred organic matter (black carbon; BC) via the analyses of benzene polycarboxylic acids and tried to identify black N forms via X-ray photoelectron spectroscopy (XPS) in order to elucidate the contribution of pyrogenic N to total N sequestration.

The results showed that total N- and amino acid-N stocks in the topsoils were significantly larger in paddy-managed Andosols and Chinese Alisols than in their non-paddy counterparts. In other soils, however, paddy management did not lead to elevated proportions of total N and amino acid-N. The N storage in peptide-bound amino acids went along with elevated contents of bacteria-derived D-alanine and D-glutamic acid, as well as with increasing stocks of DCB extractable pedogenic oxides. In order to be able to track specifically the N sequestration into the residues of bacteria and fungi, I analyzed amino sugars as respective marker compounds. Across all soils under study, the stocks of DCB extractable oxides showed a positive correlation to stocks of microbial residue ($p<0.01$, $R^2= 0.60$), whereas paddy management did not continuously led to accumulation of microbial residues. Therefore, I conclude that the mineral assemblage of soils modulates the degree at which microorganisms contribute to the N sequestration under both paddy and non-paddy management, therewith supporting the overall hypothesis of my thesis that the mineral assembly drives the overall enrichment of specific N forms in soil.

The traditional burning of straw on paddy fields led to an enrichment of BC. However, the proportion of pyrogenic organic N (black N, BN) in the soil was hardly or not detectable by XPS analysis, although a pre-test in the laboratory showed that after the combustion of rice straw the content of BN can rise to more than 50% of the total N. BC and BN did not show any correlation to soil properties such as clay content and pedogenic oxides.

Therefore, I conclude that BN from burnt crop residues does not significantly contribute to N sequestration in rice soils.

In summary, the results showed that significant amounts of N sequester in aged amino acids and microbial residues, whereas I did not find significant amounts of pyrogenic N.

The N sequestration was largely determined by the mineral assemblage of the parent material and, contrary to what was assumed, was largely independent of paddy management.

ZUSAMMENFASSUNG

Stickstofffestlegung in Böden mit und ohne Nassreisanbau aus unterschiedlichen Ausgangssubstraten

Böden unter Nassreisanbau weisen eine niedrige Stickstoff-(N)-Nutzungseffizienz auf. Man weiß, dass in Nassreisböden signifikante Mengen von N in gealterten Aminosäuren, mikrobiellen Rückständen oder pyrogenem N festgelegt werden können. Das Ziel meiner Doktorarbeit war es, diese Formen der N Sequestrierung in verschiedenen Bodentypen zu identifizieren und zu klären, inwieweit die Eigenschaften des Ausgangssubstrates und/oder die Bodenbewirtschaftung des Nassreisanbaus die N Sequestrierung beeinflussen.

Meine Hypothese war, dass die Auswirkungen des Nasseisanbaus auf die N-Sequestrierung im Boden abhängig sind von der Mineralzusammensetzung des Ausgangssubstrats.

Meine spezifischen Forschungsfragen waren: (i) führt Nassreisanbau zu einer N-Sequestrierung in Peptidbindungen, (ii) tragen Bakterien oder Pilze zu einer erhöhten N-Sequestrierung in Nassreisböden bei, und (iii) inwieweit ist im Reisanbau traditionell durchgeführte Strohverbennung verantwortlich für die N-Sequestrierung in verkohlter, stabilisierter organischer Substanz?

Um diese Forschungsfragen zu beantworten, habe ich Reisböden und angrenzende Ackerböden ohne Nassreisanbau aus verschiedenen Bodengruppen (Vertisole, Andosole, Alisole und ein Gleysol/Fluvisol-Paar) in Zentren der Reisproduktion (Indonesien, China und Italien) beprobt. Ich habe N-Biomarker wie Aminozucker und Aminosäure-Enantiomere verwendet, um zu klären, ob die N-Sequestrierung in Peptiden und mikrobiellen Rückständen in Reisböden stärker ist als in Nichtreisböden. Ausgewählte Proben wurden mit Dithionit-Citrat-Bicarbonat (DCB) vorextrahiert, um die Rolle reaktiver pedogener Oxide für die Sequestrierung, Herkunft und Zusammensetzung des gebundenen N in Aminosäuren (Aminosäure-N) besser zu verstehen. Zusätzlich analysierte ich pyrogenen organischen Kohlenstoff (Black Carbon; kurz BC) via Analyse von Benzolpolycarbonsäuren und versuchte, pyrogene N-Formen via Röntgen-Photoelektronenspektroskopie (XPS) zu identifizieren, um den Anteil von pyrogenem N im Boden festzustellen.

Die Gesamt-N- und Aminosäure-N-Gehalte unter Nassreisanbau waren nur bei Andosolen und Alisolen in China signifikant größer als bei den Vergleichsböden. In allen anderen Böden war kein Effekt von Nassreisanbau zu erkennen. Die N-Sequestrierung in peptidgebundenen Aminosäuren ging einher mit erhöhten D-Enantiomeranteilen in Alanin und Glutaminsäure (Biomarker für bakterielle N Sequestrierung) sowie mit steigenden Gehalten an DCB-extrahierbaren pedogenen Oxiden in den Böden. Um die N-Sequestrierung in Rückständen von Bakterien und Pilzen gezielter verfolgen zu können, habe ich Aminozucker als Marker für mikrobielle Rückstände analysiert. In allen untersuchten Bödentypen korrelierten die Vorräte von DCB extrahierbaren pedogenen Oxide mit den Vorräten an mikrobiellen Rückständen ($p<0.01$, $R^2= 0.60$), wohingegen der Anbau von Nassreis nicht immer zur Anreicherung von mikrobiellen Rückständen führte. Daher komme ich zu dem Schluss, dass die Mineralzusammensetzung der Böden den Grad moduliert, in dem Mikroorganismen zur N-Sequestrierung sowohl unter Nassreis- als auch unter anderen Nutzungen beitragen, was die Gesamthypothese meiner These unterstützt, dass die Ausgangssubstrate die N-Sequestrierung maßgeblich mitbestimmen.

Die traditionelle Strohverbrennung im Nassreisanbau führte, gegenüber den Vergleichsböden, zu einer Anreicherung von BC. Allerdings war der Anteil pyrogener organischen N (Black Nitrogen, kurz BN) im Boden mittels XPS-Analyse kaum bis nicht nachweisbar, obwohl ein Vortest im Labor ergab, dass nach der Verbrennung von Reisstroh der Gehalt an BN auf über 50% des Gesamt-N steigen kann. BC und BN zeigten auch keine Zusammenhänge zu den Bodeneigenschaften, wie z.B. Tongehalt und pedogenen Oxiden.

Daher schlussfolgere ich, dass BN aus verbrannten Ernterückstände nicht signifikant zur N Sequestrierung in Reisböden beiträgt.

Zusammenfassend kann ich zeigen, dass signifikante Mengen von N in gealterten Aminosäuren, und mikrobiellen Rückständen festgelegt werden konnten, jedoch fand ich keine signifikanten Mengen an pyrogenen N. Die N-Sequestrierung wurde maßgeblich durch die Mineralzusammensetzung des Ausgangsubstrates bestimmt, doch anders als vermutet, war sie weitestgehend unabhängig vom Nassreisanbau.

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LIST OF ABBREVIATIONS

^{14}C	Radiocarbon
a	Years
AA	Amino Acids
Al	Aluminum
Ala	Alanine
AS	Amino Sugars
Asp	Aspartic acid
BPCAs	Benzene polycarboxylic acids
B3CAs	Benzene-Tricarboxylic acids: ∑ Hemimellitic, Trimellitic, Trimesic acids
B4CAs	Benzene-Tricarboxylic acids: ∑Pyromellitic, Mellophanic, Prehnitic acids
B5CA	Benzenepentacarboxylic acid
B6CA	Mellitic acid
BC	Black Carbon
BN	Black Nitrogen
C	Carbon
CF_3CO_2H	Trifluoroacetic acid
CH	Hydrocarbon
COOH	Carboxylic acid
char-BC	Charcoal residue
CO_2	Carbon Dioxide
C_{org}	Organic Carbon
D-enantiomers	Right-handed amino acid
DFG	Deutsche Forschungsgemeinschaft
$\delta^{15}N$	Standardized parameter for the abundance of the ^{15}N isotope
EA	Elemental analyzer
e.g.	exempli gratia
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
FID	Flame Ionization Detector
Fig.	Figure
Gal	Galactosamine
GC	Gas-Chromatograph
Glu	Glucosamine

H	Hydrogen
H_2O	Water
HCL	Hydrochloric Acid
HI	Hydrogen index
HNO3	Nitric Acid
i.e.	it est
IRMS	Isotope Ratio Mass Spectrometry
L-enantiomer	Left-handed amino acid
Lys	Lysine
M	Mol
Mn	Manganese
MS	Mass Spectrometry
MurA	Muramic acid
N	Nitrogen
NMR	Nuclear Magnetic Resonance
NP	Non-paddy
N_t	Total nitrogen
O	Oxygen
OM	Organic Matter
p	Level of probability
P	Paddy
PFPA	Pentafluoro-Propionic acid
R^2	Coefficient of Determination
SD	Standard Deviation
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SON	Soil Organic Nitrogen
Tab.	Table
WRB	World Reference Base of Soil Resources

I.

GENERAL INTRODUCTION

1 Rationale

As of August 2016, the world population was estimated at 7.4 billion (UN 2015). The United Nations estimate it will further increase to 11.2 billion until the year 2100. Rice is the staple food for over 50% of the world population and provides 20% of the world's dietary energy supply (FAO 2004). More than 80% of the world rice production is carried out under wet rice field management, which includes ploughing and puddling of the soil under submergence and followed draining of fields, processes that initiate the development of so-called anthropogenic paddy soils (Datta 1981; WRB 2014). One major problem in these paddy soils is the low nitrogen (N) use efficiency (Cassman et al. 1996; Kögel-Knabner et al. 2010) caused by high N_2O emissions, NO_3^- leaching and N sequestration in the soil (Cassman et al. 1996b; Geisseler et al. 2010a, Roth et al. 2011, Jiang et al. 2013).

Nitrogen (N) is a major nutrient, controlling the plant growth. As a limiting growth factor, the concentration and availability of N is closely related to biological productivity (Knicker 2007). Due to exponential rise of the world population and the mentioned role of rice, the productivity and economic efficiency of rice cultivation is crucial for the nutrition of mankind.

Long-term paddy management is considered to accumulate soil organic matter (SOM) (Kögel-Knabner et al. 2010). More than 90% of soil N is bound in SOM (Stevenson 1982), defined as soil organic nitrogen (SON) General in soils, the N accumulation takes place at a scale of days to decades (Amelung et al. 2006) and many different stabilization processes take part (Schulten und Schnitzer 1997). Major pathways are ageing of SON bound in peptides, and incorporation and accompanied sequestration of SON into soil microbes and their residues (Geisseler et al. 2010). Parts of the dead microbial biomass, with incorporated SON, get preserved in the soil and enable N ageing. Presumably this preservation is supported by organo-mineral interaction (Lützow et al. 2006, Schrumpf et al. 2013).

Another often underestimated SON sequestration pathway may be accumulation of pyrogenic organic N, which derives from incomplete combustion of plant and litter peptides (Knicker 2007). The traditional burning of rice straw favors accumulation of soil organic carbon (SOC) (Lehndorff et al., 2014). However, the effect of straw burning on SON accumulation in paddy fields is yet unknown.

Several studies showed that long-term paddy management influences morphological, biological, physical and chemical properties in soils (e.g. Huang, 2014, Kögel-Knabner et al. 2010). Presumably this such redox related processes are responsible also for enhanced N sequestration in paddy soils. However, I am not aware of any study which determined the influence of soil-specific mineral assembly of the parent material on SON sequestration under paddy management. Thus, I focused on paddy soils which formed from different parent materials (Alisols, Andosols, Vertisols, Gleysols), to elucidate if their SON sequestration is influenced by paddy management. Adjacent non-paddy soils served as references. I used N biomarker, like amino sugars and amino acid enantiomers, to elucidate whether SON sequestration in peptides and microbial residues is stronger under paddy compared to non-paddy management. Additionally, I analyzed charred organic matter (black carbon) and tried to identify black N forms via X-ray photoelectron spectroscopy (XPS) to elucidate the portion of pyrogenic SON sequestration.

2 State of the art

2.1 The importance of rice

Rice is one of the three leading food crops in the world. Rice, wheat and maize supply more than 50% of all calories consumed by the entire human population. Of these three major crops, rice is the most important food source for people in low- and lower-middle-income countries (FAO 2015). In Asia, rice consumption is very high, exceeding 100 kg per capita annually in many countries. Since the green revolution in the 1960s the total global rice consumptions raised from 150 to over 500 million tons per year (FAO 2004, 2016). Typically rice production is performed under wet rice field management which has shaped a specific type of wetland soil (WRB, 1998) – the so called paddy soils.

2.2 Paddy soils and their characteristic biogeochemistry

According to the World Reference Soils Base (WRB 1998), paddy soils are classified as Hydragric Anthrosols and can originate from any soil type. They develop specific biogeochemical characteristics due to frequent submergence and drainage of the rice field, tillage under flooded conditions (the so called puddling), high liming and fertilization (Gong 1983, Kirk, 2004). In consequence, the paddy soils develop a characteristic soil profile (Fig.I 1). During seasonal flooding, a layer of standing water (W) covers the soil, followed by a thin oxic zone (Apo) caused by oxygen release from rice roots. Due to submergence, the topsoil is developed as an anthraquic horizion (Ap). The upper part is the reduced puddled layer (Arp) which is densely rooted and rich in organic matter.

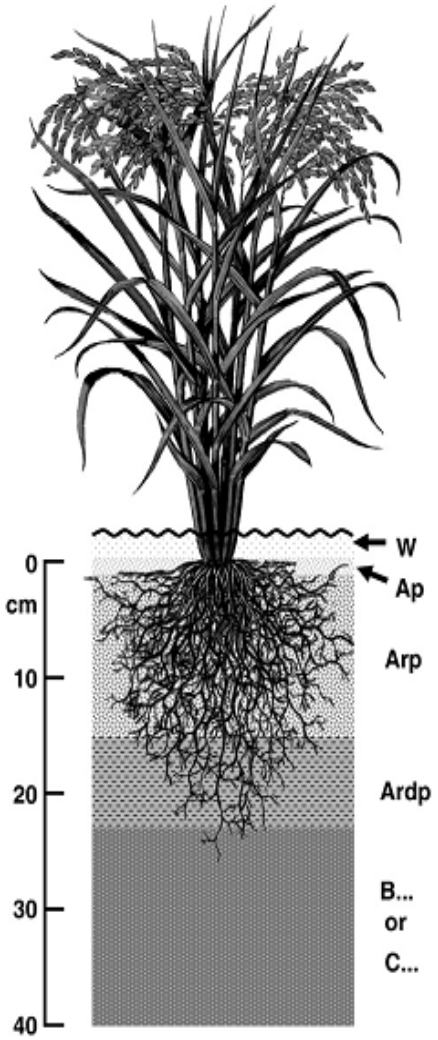

Fig.I 1: Typical horizon sequence of a rice paddy soil (horizon designation according to FAO, 2006) (*Kögel-Knabner* et al., 2010**)**

The puddling with tractors or water buffalos leads to the formation of a plough pan (Ardp) below the puddled layer (Kögel-Knabner et al. 2010). The compacted plough pan decreases

infiltration rates and ensures submergence. The density of the plough pan depends on the duration of rice cropping and puddling intensity (Janssen und Lennartz 2007). Those hydraulic properties control the water infiltration into the underlying subsoil (B). Flooding and fertilization practices, as well as straw management, differ in dependence of social, economic and climatic conditions between regions (Fusi et al., 2014).

The changing redox conditions strongly affect organic and mineral soil components of the soils (Kögel-Knabner et al. 2010b). Under oxic conditions, microorganisms use oxygen (O_2) as an electron acceptor for respiration. Under anaerobic conditions alternative electron acceptors are utilized to obtain energy following the decreasing redox potential (Eh). Subsequently, nitrate (NO_{3-}), iron (Fe^{3+}) and sulfate (SO_{4-}) get reduced. Due to this, paddy soils are known as emitters of greenhouse gases like methane and nitrous oxides (Cai et al. 1997; Hua et al. 1997) and were mentioned in the IPCC (Intergovernmental Panel on Climate Change) report as contributors to climate change (Smith et al. 2007). On the other hand, paddy soils were listed in the Kyoto protocol as C and N sink. SOM accumulates because of high inputs of plant residues and organic fertilizers (Neue et al. 1997) and presumably the frequent change of redox conditions decreases decomposition of SOM (including SON) due to reduced microbial degradation processes during anaerobic phases (Kögel-Knabner et al. 2010b). Ensuing SOM stabilization occurs by occlusion into stable aggregates, adsorption to clay or organo-mineral interaction, e.g. iron oxides (Olk et al. 1996; Schmidt-Rohr et al. 2004; Kögel-Knabner et al. 2008 ; Mikutta und Kaiser 2011; Said-Pullicino et al. 2015). However, paddy management appears on a variety of soil groups with different pedogenic properties and the soil groups may obtain different sensitivities to paddy management (e.g. Huang et al. 2015). To which extend the properties of the original parent material are crucial for SOM accumulation in paddy soils is still unknown. The SOM included over 90 % of nitrogen (N).

2.3 Soil organic nitrogen (SON) in paddy soils

N dynamics are one of the most complex biogeochemical cycles in agriculture (Said-Pullicino et al., 2014) and beside C, N is the most important element in agricultural systems (Schulten und Schnitzer 1997).

In soils approx. 40-50% is proteinaceous material (proteins, peptides, and amino acids), 5-10% are amino sugars, 20-35% are heterocyclic N compounds, 10-19% NH_3, whereas 1/4 of the NH_3 is fixed as NH_4^+ (Schmidt-Rohr et al. 2004, Schulten und Schnitzer 1997; Knicker 2007).

Main input pathways into soil are via biological N fixation, irrigation, precipitation, OM input (e.g. plant residues), mineral and organic fertilization. N losses occur in form of plant uptake, volatilization, denitrification, leaching and runoff (Stevenson 1982, Cassman et al. 1996a Brodowski et al. 2005, Jiang et al. 2013). Further N transformation processes in soils are nitrification, ammonification and sequestration (Stevenson 1982; Kyuma 2004, Jiang et al. 2013). Major problem in paddy soils is the high SON sequestration (Said-Pullicino et al. 2014) and resulting low fertilizer use efficiency (Zhao, 2009). The SON sequestration processes and determining factors in paddy soils are still unclear. Furthermore we are not aware of any study that determined the specific impact of original soil properties (Fe, allophanes, clay) on N sequestration in paddy soils.

2.3.1 SON sequestration in paddy soils

N sequestration processes operate at scales of decades and involves the bonding of ammonia to lignin-phenols (Schmidt-Rohr et al., 2004), ageing in peptides (Amelung et al. 2006), microbial SON immobilization by bacteria and fungi (Ding et al. 2011) and pyrogenic SON input due to the traditional burning of crop residues favors an increase of SON stability (Knicker 2007).

Through chemical and physical binding, SON associates with organic and inorganic substances, such pedogenic oxides, clay minerals and soil aggregates (Constantinides und Fownes 1994, Chantigny et al. 1997, Cheshire et al. 2000, Amelung et al. 2001, Mikutta et al. 2010, Chen et al. 2014). Such conserved SON can even persist in the soil for centuries to millennia (Amelung 2003, Mikutta et al. 2010).

It is assumed, that anaerobic conditions in paddy soils, i.e. chancing redox potential, favor N ageing in peptides and high microbial immobilization of N via organo-mineral interactions (Kögel-Knabner et al., 2010; Olk et al., 2007; Said-Pullicino et al., 2014). According to this, previous studies suggested that paddy soils sequester significant amounts of SON in a) peptides, b) microbial residues and c) charred organic matter (Roth et al. 2011, Roth et al. 2013, Lehndorff et al. 2014, Said-Pullicino et al. 2014).

a) SON sequestration in peptides

Amino acids contribute up to 45% to SON contents in soil (Stevenson, 1982, Amelung 2003) and are the basic structure of proteins and peptides, which originate from plants, microorganism and other living tissues. These peptide bound amino acids are involved in N turnover and contribute an important energy source for microorganisms (Senwo et al. 1998).

Amino acids are found in two enantiomeric forms: left handed L- and right handed D-form (Bada, 1984). The composition of D/L-enantiomers and different physical-chemical properties of the R-groups of amino acids can help to elucidate N cycling processes. Living cells almost consist of L-enantiomers (Jonas, 2005). There are two main pathways for the formation of D-enantiomers. First, microbes are known to produce D-enantiomers and incorporate them into their cells walls and therefore protect them from cell-own protease (Schleifer and Kandler, 1970, Amelung, 2003). Due to this, D-enantiomers are suitable biomarkers for bacterial N sequestration; particularly D-alanine and D-glutamic acid (Amelung, 2003). Second, after cell death a very slow abiotical racemization takes part converting aspartic acid and lysine from L- into D-enantiomers (Bada, 1984). This process can be used as marker for N aging. The assessment of the protein age give us an important clue of the residence time of SON. Amelung (2003) found changes in bacterial N sequestration under different climates, cropping systems and duration of cropping. In a greenhouse experiment with 13C labeled paddy soils, Roth (2011) found a reduced rhizodeposition in older paddies and related this to a better adapted microbial community.

Thirdly, the R-groups of amino acids can be classified by their general physical-chemical properties of their R groups. The main groups are polar/neutral, nonpolar (hydrophobic), basic and acidic amino acids. Specific amino acid R-group composition is an indicator for soil properties (Schulten and Schnitzer, 1998) and differ between cultivation and management practices (Senwo, 1998, Friedel, 2002, Martens 2003). Up to now a potential change of amino acids R-group composition in paddy soils compared to non-paddy soils is not well studied. If some amino acid groups are enriched or depleted is also not studied yet. Further the influence of the parent material on SON sequestration in specific amino acid R-groups is unknown.

In summary, determination of amino acid enantiomers allows us a deeper view into the dynamics of the SON cycle. However, actually no studies were available on the influence of paddy management on SON sequestration and accompanied ageing in soil peptides and the assumed influence of parent material as stakeholder for organo-mineral interactions.

I assume that paddy management does not only promote the sequestration of soil N in amino acids, but that the degree of the SON storage differs among different major reference soils and their site-adapted paddy management practice.

b) SON sequestration in microbial residues

The build-up of microbial biomass is a key factor in SOM accumulation and stabilization (Miltner 2012). Microbial community transforms SOM and SON in fungal and bacterial cell walls within proteins in forms of amino sugars (up to 10% of SON) or amino acids (up to 45% of SON) (Guggenberger et al., 1999; Roth et al., 2011).

The incorporated N becomes partly unavailable for plants (Geisseler et al. 2010). Immobilized N, however, can be remineralized again. The turnover rate depends on different factors. Ammonification is enhanced by aeration, high temperatures and a high amount of clay with high cation exchange capacity. It is furthermore influenced by the composition of OM (e.g. C/N ratio), pH and soil moisture (Kyuma, 2004, Blume et al., 2010, Kögel-Knabner et al., 2010, Ding et al., 2015).

To get a closer view into the microbial community I used amino sugars as biomarker for microbial N sequestration. Amino sugar allow the differentiation between fungal and bacterial residues in soil. These microbial groups are the largest functional groups in soils, whereas archaea and protozoa contribute only 1–2% (Gattinger et al. 2002). The most important amino sugars are glucosamine, galactosamine, muramic acid and mannosamine (Amelung et al. 2001).

Glucosamine mostly originates from fungal chitin and muramic acid uniquely derives from bacteria (Parson 1981). Fungi and bacteria were separated because of their different functional behavior in soils. Fungi favor acidic soils with low available nutrients, recalcitrant organic materials, and high C/N ratios and use organic substance more efficiently than bacteria (Holland und Coleman 1987; Blagodatskaya und Anderson 1998). Due to this the population of fungal hyphae grow slowly, but are more resistant against further microbial decomposition than bacterial polymers (Webley und Jones 1971; Guggenberger et al. 1999). In nutrient-rich soils the bacterial community is more pronounced. They prefer more easily decomposable substrates, which promote a rapid growth, a fast cycling of nutrients and allow a quick response to environmental changes (Ingwersen et al. 2008; Amelung et al. 1999; Roth et al. 2011). High nutrient contents are often combined with higher organic matter input by plants, which, in turn, promoted by increasing land use intensity, like fertilization, tillage, crop variant and rotation (Frey et al. 1999; Högberg et al. 2003). Paddy soils are usually very intensive landuse systems with e.g. high fertilization applications and deep ploughing (so called puddling). Presumably this favors bacterial more than fungal growth. According to this Roth et al. (2011) determined a higher bacterial N sequestration in paddy soils compared to adjacent non-paddy soils.

I assume that the slower builded-up microbial residues (due to decreased SOM decomposition) became protected from further decay thru mineral-organo interactions which were promoted by changing redox potential in paddy soils. As a result paddy soils should sequester more nitrogen in microbial residues compared to other cropping systems. I hypothesizes that the paddy management leads to higher proportion of bacteria (vs. fungi) related to non-paddy managed soils due to better management adaptability (like waterlogging, puddling and intensive fertilization). Further, it is unknown how far the paddy management on different soil groups changes the N sequestration in microbial residues, and especially in bacterial residues is so far unknown. Especially the impact of soil properties (e.g. soil minerals) on further conservation of SON is still unknown. I hypothesized that the mineral assembly of the original parent material is crucial for long-term N sequestration in microbial decomposed soil organic nitrogen via mineral-organo interactions.

c) SON sequestration in charred organic matter

Paddy soil crop rotations frequently receive additions of considerable amounts of burned rice straw. In Chinese paddy management this tradition leads back to at least 6000 years (Cao et al., 2006). Thus, elevated black carbon concentrations were found in recent and buried top soils of the paddy soils of the Cixi area, China (Lehndorff et al., 2014), even if the contribution to total SOC did not exceed 10%. Yet, the incorporation of burned carbon structures should also include heterocyclic, aromatic N forms (e.g. Knicker, 2007). Mikutta et al. (2010) found that even 18-34% of N was aromatic N in aerated soils of Hawaii, which was likely a result of pyrogenic OM input. In previous studies found that at least about 50% of paddy´s SON was not related to microbial-bound SON (Roth et al., 2011) and proteinaceous amino acid SON (unpublished data). Hence, I assume that may thus also be a significant contribution of black N to stable SON stocks in paddy fields.

Further, there is evidence that the turnover of BC in soils also depends on the soil mineral assembly. I hypothesis that overall BC storage in soil relates to paddy management and the abundance of reactive mineral phases such as Fe and Al oxides, and clay-sized minerals. Parallel to BC, black nitrogen (BN) should accumulate in soil.

If paddy management leads to accumulation of N in heterocyclic, aromatic forms may be revealed by X-ray photoelectron spectroscopy (XPS; Abe et al., 2005; Mikutta et al., 2010). For example, Ding et al. (2014) related the aromatic N peak in the XPS N 1s spectra to black N in water samples and found a coupling between “dissolved” black N and BC. Here, I tested

whether this method is applicable to rice char and soil, and if a correlation of BC to aromatic N in soil exists.

2.4 Long-term N sequestration via organo mineral interactions

Stabilization of OM by interactions with minerals is of increasing importance for long-term N sequestration. Upon frequent tillage, however, and possibly even more during the puddling in rice management followed by dryland cropping, interactions of SOM with soil minerals influences increasingly the fate of SOM (Lützow et al., 2008). Flessa et al. (2007) suggested that there was an average turnover of 200 years for mineral-bound OM in soils cropped with maize, which is 4 times more than its turnover of SOC in the bulk soil. Transferred to SON, it should thus be possible to differentiate short- and long-term SON cycles when differentiating whether this SON is bound to minerals or not.

The SOM maybe bound to the mineral phase by ligand exchange at edge sites of phyllosilicates and mineral surface hydroxyl groups (e.g. ferrihydrite; Kleber et al., 2005), or by polyvalent cation bridges due to the permanent loading of clay minerals (Kahle et al., 2002). Association to hydrophobic surfaces on non-charged mineral surfaces (van der Waals forces) was observed for acidic (forest) soils but may only play a minor role in paddy soils (Chenu et al., 2000; Lützow et al., 2008).

With regard to SON in peptides and microbial residues, amino acids and amino sugars can be bound to minerals by cation exchange reactions of their amino group or by ligand exchange with metal hydroxides by their carboxyl group (Cheshire et al., 2000). The latter process dominates in clayey soils. This is in accordance with observations by Kaiser and Zech (2000) who also found a preferential binding of muramic acid to minerals via its carboxylic group rather than via its N group. Besides, amino acids can also be bound to the interlayer spaces in phyllosilicates and remain there for millenia (Cheshire et al., 1992). Soils with different parent materials may thus exhibit different stabilization mechanisms for amino acid N.

The suggestion that different parent materials contribute to different storage of SON was, e.g., investigated by Moritz et al. (2009). They reported for tropical forests that the sedimentary substrates preserved higher amounts of amino sugars than metamorphic, ultrabasic substrates and linked this to changes in iron oxide crystallinity in the subsoil. Another study on the mineralogical impact on organic nitrogen sequestration was later on published for a chronosequence of aerated, volcanic soils in Hawaii (Mikutta et al., 2010). The authors investigated amino sugars, amino acids and the ^{14}C content of mineral-bound SON and could

show a strong initial association of acidic amino acids to metal(hydro-)oxides, organic precipitates and variable-charge minerals (intermediate weathering, 20-400 yrs.). In the final weathering stage of the chronosequence (1400-4100 yrs.) with well-crystalline mineral phases in the soil, less SON was preserved and particularly acidic amino acids where depleted while aromatic N dominated. The mineral-associated N in the crystalline topsoils seemed to be actively involved in the nitrogen cycle.

Under paddy management, i.e. frequent submergence, SOC and N concentrations are generally higher in the surface soil horizons compared with aerobic agricultural soils (e.g. Olk and Cassman, 1996; Li et al., 2005). The redox cycle may lead to increasing precipitation of poorly crystalline oxides that preferentially preserve OM in dry phases (Cheshire et al., 2000; Kleber et al., 2005). In addition, microbial cycling rates may be directly affected by mineral surfaces. Incubating forest litter with different minerals, for instance, suggested that litter turnover and microbial synthesis of amino sugar N was inhibited in the presence of Al oxides and, to a lesser degree, by Fe oxides but enhanced by Mn oxides (Miltner and Zech; 1999; Amelung et al., 2001); these processes being more pronounced for fungal amino sugars than for the bacterial ones. Whether these findings imply, for instance, that also in Andosols the turnover of paddy SON is reduced and driven by bacteria compared to Alisols and Vertisols, respectively, is still unclear.

3 Objectives

Paddy management was shown to influence soil properties, such as OM contents and turnover. This suggests that N dynamics in paddy soils differ from other, aerobic cropping systems, however, it was not yet shown if this can be transferred to different soils of the world. Particularly the N sequestration in peptides, microbial and pyrogenic residues is enhanced in paddy soils. However, it is still unclear if there are differences related to the mineral assembly of the soil parent material. In rice production areas in Asia Vertisols, Alisols and Andosols represent a huge range of soil properties from clay-rich soil to soils with high portions of reactive Fe and Al mineral phases. In Europe, Italy is the largest rice producer and mostly Fluvisols in the Po valley were under paddy management.

Therefore, I compared five paddy and non-paddy soil pairs from different parent materials to answer the following research questions:

i. Does paddy management lead to N sequestration in peptide bonds?

Here, I hypothesized that paddy management promotes the storage of SON, especially via sequestration in amino acids. The degree of SON and amino acid storage differs among different major reference soils under paddy management. To test these hypotheses, I sampled triplicates of five pairs of paddy and non-paddy cropped soils, developed on different parent materials, and I assessed soil N stocks, amino acid-N stocks, and the amino acid enantiomer composition. In order to elucidate the interactions between SON storage and the amount of pedogenic oxides, I repeated amino acid enantiomer analyses after treatment of samples with dithionite–citrate–bicarbonate (DCB).

ii. Do bacteria or fungi promote high microbial N sequestration in paddy soils?

I assumed that paddy management leads to higher portions of immobilized SON in bacterial cell wall residues (vs. fungal SON) compared with non-paddy managed soils due to better management adaptability of bacteria (like waterlogging, puddling and intensive fertilization). Therefore, I assessed soil organic carbon (OC) stocks, N stocks and δ15N isotope composition and combined this with an estimation of bacterial and fungal residue-N in soil via amino sugar analysis in paddy and adjacent non-paddy soils developed from major reference soil groups.

iii. To which extent can N sequestration in paddy soils be assigned to the input of charred organic matter?

Large amounts of N in soils exist in unidentified forms. Here, I assessed the storage of C and N in pyrogenic forms. The accumulation of pyrogenic N in soil due to inputs of black nitrogen (BN) is not yet well understood, I aimed to detect parallels in soil black carbon (BC) and BN contents. I elucidated the possible effects of paddy versus non-paddy management, and different major reference soil groups on the storage of BC via BPCA analysis. X-ray photoelectron spectroscopy (XPS) should reveal if straw burning in paddy management led to accumulation of N in heterocyclic, aromatic forms.

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II.

NITROGEN SEQUESTRATION UNDER LONG-TERM PADDY MANAGEMENT IN SOILS DEVELOPED ON CONSTRATING PARENT MATERIAL

Modified on the basis of:

Nitrogen sequestration under long-term paddy management in soils developed on contrasting parent material

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1 Introduction

Soils under submerged rice management, i.e., paddy soils, may promote nitrogen (N) sequestration (e.g. Kögel-Knabner et al., 2010; Cucu et al., 2013; Kölbl et al., 2014). Nevertheless, paddy soils exhibit a low N fertilizer use efficiency (Cassman et al., 1996; Zhao et al., 2009), and low plant availability of the residual N (Olk et al., 1996; Schmidt-Rohr et al., 2004; Jiang et al., 2013). The latter is due to efficient sequestration of N in organic forms (Constantinides and Fownes, 1994; Schmidt-Rohr et al., 2004; Roth et al., 2011; Jiang et al., 2013). The N sequestration takes place at a scale of days to decades (Roth et al., 2011), and likely involves bondings of ammonia to lignin-phenols (Schmidt-Rohr et al., 2004) as well as microbial N immobilization by bacteria and fungi (Roth et al., 2011; Said-Pullicino et al., 2014). However, the level of N sequestration is highly variable among different paddy soil units, and the underlying processes have not yet been not fully understood. In topsoil, most N is organically bound.

A large part of this soil organic nitrogen (SON) could be assigned to amino acids (AA), which usually comprise up to 40% of SON (Schulten and Schnitzer, 1997; Amelung, 2003). Amino acids are the basic structures of proteins and peptides. They may originate from plants, microorganisms, and other living tissue. They are important energy sources for microorganisms (Senwo and Tabatabai, 1998; Geisseler et al., 2010). Several studies showed that amino acid composition differs among soils under different management practice (Senwo and Tabatabai, 1998; Friedel and Scheller, 2002; Martens and Loeffelmann, 2003). Analyses of amino acid composition and their charge distribution (polar/neutral, nonpolar (hydrophobic), basic, and acidic amino acids can help to elucidate changes in the cycling of SON in paddy soils (Schulten and Schnitzer, 1997), particularly, when assessment of amino acid enantiomers is included (Amelung et al., 2006).

Amino acids namely exist in the left handed (L-) and right handed (D-) enantiomeric form (Bada, 1985). Living cells almost exclusively contain L-enantiomers. There are, however, two main pathways for the formation of peptide-bound D-enantiomers. First, microorganisms produce specific D-enantiomers and incorporate them into their cells walls in order to protect them from the cells' own proteases (Schleifer and Kandler, 1970; Poinar et al., 1996). These D-enantiomers, particularly D-alanine and D-glutamic acid, are suitable biomarkers for bacterial N sequestration processes (Pelz et al., 1998; Amelung, 2003). Second, after the death of cells, slow racemization transforms L- into D-amino acids as, e.g., shown for teeth and bones (Bada, 1985; Maroudas et al., 1998) and for buried soil or sedimentary organic matter (Kimber and Hare, 1992; Amelung, 2003). The respective D-amino acids then serve as

markers for relative SON aging, here, particularly D-aspartic acid and D-lysine (Brodowski et al., 2005; Amelung et al., 2008). It is, thus, reasonable to assume that biogeochemical processes during paddy soil formation will also affect microbial N turnover (Marumoto, 1984; Matsumoto and Ae, 2004) and lead to an ageing of AA-N.

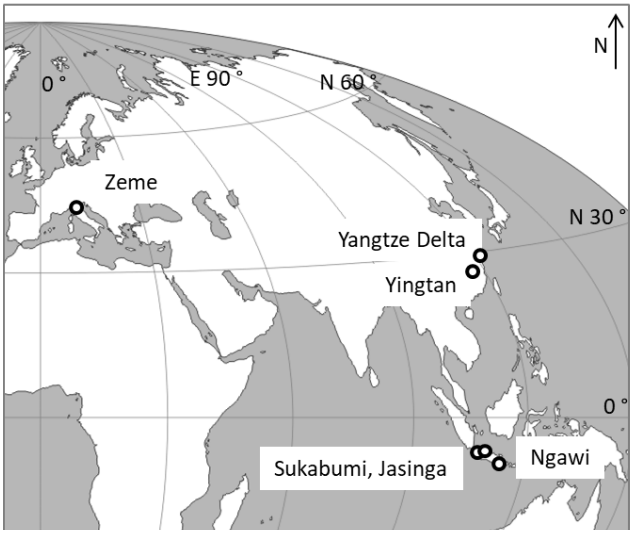
Much of the organic matter in soil is bond to oxides and clay minerals (Lützow et al., 2006; Schrumpf et al., 2013), which are thus considered as main stabilizing agents (Kögel-Knabner et al., 2008). Paddy soils, however, are prone to frequent redox cycles (Kögel-Knabner et al., 2010): Fe oxides undergo reductive dissolution under anoxic and re-precipitation under oxic conditions. As result, the contents of poorly crystalline Fe oxides (determined as oxalate-extractable Fe_o) increased and those of crystalline Fe oxides (Fe_c=dithionite-citrate-bicarbonate extractable Fe_d – oxalate soluble-extractable Fe_o) declined with prolonged paddy management (Zhang and Gong, 2003; Kölbl et al., 2014; Winkler et al., 2016). Pedogenic Fe oxides, however, are usually much more reactive than their crystalline counterparts (Borggaard, 1982), which may contribute to either the turnover of SON during dissolution (Kleber et al., 2005) or to its stabilization by the formation of Fe-soil organic matter (SOM) co-precipitates (Mikutta et al., 2010; Chen et al., 2014). Presumably, redoximorphic dynamics in paddy soils co-regulate N immobilization. However, we are not aware that this kind of organo-mineral interaction has been tested for paddy soils.

Here, we hypothesized that paddy management effects on the storage of AA-N relate to the soil mineral assembly. To test this hypothesis, we sampled triplicates of five pairs of paddy and non-paddy soils, formed from different parent materials, and we assessed soil N stocks, amino acid-N stocks, and the amino acid enantiomer composition. In order to elucidate the interactions between SON storage and the amount of pedogenic oxides, we repeated amino acid enantiomer analyses after treatment of samples with dithionite–citrate–bicarbonate (DCB).

2 Materials and methods

Sampling

We sampled five soils developed on contrasting parent material under paddy and adjacent non-paddy management (Fig.II 1), and described and classified them according to FAO (2006) and World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). Soils under paddy management were all classified as Anthrosols (Tab.II 2, see also chapter IV 2 for detailed soil description), however, for better clarity in the text, we name them according to the respective non-paddy soils, which were their precursors (e.g. Vertisol-derived paddy). Only the counterpart of the Fluvisol-derived paddy soil was classified as Gleysol. The paddy soils had characteristic puddled layers and were at least 100 years under paddy management (for details and soil horizon description see Lehndorff et al., 2016 and Winkler et al., 2016). The sites were located in Indonesia, Java (Andosols, Alisols and Vertisols), in China (Alisols) and Italy (Fluvisol/Gleysol) (Tab.II 1). The Indonesian sites were cropped with two rice periods per year, whereas the Chinese and Italian sites were cropped with one rice period per year. For each type of soil, three profiles (one main, two subsites) were dug down to at least 100 cm depth per paddy and non-paddy variant (pairs of paddy and non-paddy soils were selected according to similarities in parent material, all soils were at least for one century under permanent paddy or non-paddy cultivation). An exception was the site in Italy, where only one field site was available. The distance between adjacent profiles (on different plots) was about 50-300 m at each sampling site. Soil samples were taken destructively per horizons as defined according to FAO (2006). Topsoils samples represent the upper 30 cm and subsoils the 30-100 cm soil horizons (summarized from the single sampled and analyzed horizons; Tab.II 2 and Tab.VII 1 in Appendix B). In addition, we took undisturbed soil cores (100 cm^3, n=3) from each horizon for the assessment of bulk density. Soil samples of each horizon were air-dried at 40 °C, sieved to a size of <2.0 mm and ground for laboratory analysis.



Tab.II 1: Locations of the paddy and non-paddy soil sites under study.

Tab.II 2: Soil classification, sampling location, climate and crop history for Indonesia, China and Italy.

Soil type	Country	Parent material	texture	Precipitation [mm] / climate	Crop rotation	Elevation [m a.s.l.]	Coordinates
Andosol-derived paddy Escalic Hydragrick Anthrosol (Dystric)	Indonesia (Java) Sukabumi	volcanic ash, intermediate igneous (andesite)	silt-clay loam	2000-4400 / tropical (25°C)	*Oryza sativa* (rice), *Oryza sativa* , *Brassica rapa chinensis* (pak choi)	870-871	S 06°52.802'/ E 106°56.457
Silandic **Andosol** (Distric, Thixotropic)					*Manihot esculenta* (cassava), *Zea maya* (maize)	969-981	S 06°52.029'/ E 106°56.725'
Alisol-derived paddy Escalic Hydragrick Anthrosol (Dystric, Clayic)	Indonesia (Java) Jasinga	andesite	silt loam	2500-3500 / tropical (27°C)	*Oryza sativa* (rice), *Oryza sativa* , *Zea maya* (maize)	238-248	S 06°32.205'/ E 106°31.062'
Hyperalic **Alisol** (Abruptic, Humic, Clayic, Chromic)					agroforest: *Manihot esculenta* (cassava), *Musa spec.* (banana), *Leucaena leucocephala* (mimosa)	236-238	S 06°32.158'/ E 106°31.030'
Vertisol-derived paddy Hydragrick Anthrosol (Eutric, Clayic)	Indonesia (Java) Ngawi	Fluvial sediments from andesite	clay-silt clay	2000-3000 / tropical (27°C)	*Oryza sativa* (rice), *Oryza sativa* , *Nicotiana spec.* (tobacco)	77-78	S 07°26.878'/ E 111°36.599'
Pisocalcic Mollic Grumic **Vertisol** (Humic, Hypereutric, Pellic)					*Saccharum officinarum* (sugar cane)	78-80	S 07°26.691'/ E 111°36.667'
Alisol-derived paddy Escalic Hydragrick Anthrosol (Dystric)	China, Yingtan	limnic and fluvial sediments from conglomerates	silt-clay loam	1300-1900 / sub-tropical (18°C)	*Oryza sativa* (rice), *Trifolium spec.* (clover)	40-45	N 28°14.020´/ E 116°53.866´
Cutanic Hyperalic **Alisol** (Chromic)					*Arachis hypogaea* (peanuts), *Sesamum indicum* (sesame), *Ipomoea batatas* (sweet potatoes)	50-57	N 28°14.035´/ E 116°53.784´
Fluvisol-derived paddy Haplic Gleysol (*Eutric, Arenic,* Densic)	Italy, Zeme	alluvial sediment	sandy loam	700-1000 / temperate (13 °C)	*Oryza sativa* (rice), fallow	79-80	N 45°11.536´/ E 8°40.078´
Endogleyic **Fluvisol** (*Eutric Arenic)*					*Zea maya* (maize), fallow	79-80	N 45°11.555´/ E 8°40.104´

Basic laboratory analyses

For the Indonesian and Chinese sites, texture, bulk density, pH, CEC, total C and N, organic carbon (OC), as well as oxalate- (Fe_o) and dithionite–citrate–bicarbonate-extractable Fe (Fe_d) were taken from Winkler et al. (2016). Total C and N were measured in duplicate by dry combustion at 950 °C using a Vario MAX elemental analyzer (Elementar Analysensysteme, Hanau, Germany). In case of carbonates, inorganic C (IC) content was determined by dissolution of carbonates with 42% phosphoric acid and subsequent infrared detection of the evolving CO_2 (C-MAT 550, Ströhlein GmbH, Viersen, Germany). Contents of IC were subtracted from total carbon contents to obtain the organic C (OC) contents. Iron (Fe), Mn, and associated Al were extracted in duplicate by dithionite–citrate–bicarbonate (DCB) treatment according to Mehra and Jackson (1960). The remaining soil was filtered, washed

with distilled water, and then air-dried. A subset of these residues, namely one topsoil horizon sample (0-15 cm) and two subsoil horizons samples (≈30-40 and ≈40-50 cm) of each paddy and non-paddy main site pair were then subjected to the analyses of amino acid enantiomers.

Analyses of amino acids

As microorganisms are known to synthesize a variety of D-amino acids in free and water-soluble forms (Nagata et al., 1998), free amino acids were removed with 1 M HCl (12 h, 25 °C; Kvenvolden et al., 1970; Amelung and Zhang, 2001). Then, protein-bound amino acids were hydrolyzed with 6 M HCl and processed as described by Amelung and Zhang (2001). Briefly, L-norvaline was added as internal standard after hydrolysis. The hydrolysate was then filtered and dried by rotary evaporation. Purification was conducted by using cation exchange resins. Oxalic acid was used for metal removal, followed by 2 M ammonium hydroxide for amino acid elution, and then, non-soluble substances were removed by centrifugation. D-methionine was added prior to derivatization to determine the recovery of the internal standard. For derivatization to N-pentafluoropropionyl isopropyl esters, dried samples were esterified with 4 M HCl in isopropanol. Then, dichloromethane and pentafluoropropionic anhydride were added to the dry amino acid-ester hydrochlorides. The N-N-pentafluoropropionyl-isopropyl-ester derivatives were measured on a gas chromatograph-mass spectrometer (GC-MS) (Agilent 5971, Agilent GmbH, Böblingen, Germany) using a chiral column (CP-ChiraSil-L-Val CP7495, Agilent, 25 m, I.D. 0.25 mm, film 0.13 µm). For further details on the method and the GC-MS temperature program, see Amelung and Zhang (2001). On average, > 80% of L-norvaline was recovered after sample processing. The main soil profiles were characterized for their contents of D-/L-amino acids up to 100 cm depth for each single horizon. For the two subsites, these analyses were restricted to the first, second and fifth classified soil horizon. As these data corresponded well to the data of the main sites, we linearly interpolated the values for the third and fourth horizon for calculating the mean

amino acid contents of the two subsites. The interpolation changed the average amino acid stocks per site by less than 6% relative to the calculation of means where we used measured data only.

Statistical analysis

Data were statistically compared using Sigma Plot 13 (Systat Software Inc.; San Jose, USA) and STATISTIKA 8.0 (Stat-Soft, Inc.). Differences in total N stocks, AA-N stocks and D/L ratios of alanine, glutamic acid, aspartic acid and lysine of the top- and subsoils were tested between soils (ANOVA) and for the combined effect of paddy and non-paddy management of the different soil groups (MANOVA).

Linear and multiple regression were used for testing correlations between DCB Fe-, Al and Mn contents, pH, CEC, particle size distribution with bulk soil amino acid contents and D/L composition.

3 Results

N stocks in paddy and non-paddy soils

The total N stocks down to 100 cm depth varied from 5.0 to 14.6 t ha^{-1} in paddy and non-paddy soils. Andosols had highest total N stocks with 14.6 t ha^{-1} under paddy, and 10.8 t ha^{-1} under non-paddy management, of which the topsoils (0-30 cm) contained 9.4 and 6.7 t N ha^{-1} for the paddy and non-paddy management, respectively (Fig.II 1). The lowest N stocks under paddy management were observed in the Vertisols with 6.1 t ha^{-1} (100 cm soil depth; 2.7 t ha^{-1} in the topsoil). The lowest N stocks under non-paddy management were observed in the Chinese Alisols (5.0 t ha^{-1} for 100 cm depth; 2.1 t ha^{-1} for the topsoil). Hence, management effects on total N storage in topsoils (0-30 cm) was site-specific: larger topsoil N stocks occurred under paddy management for Andosols ($P < 0.05$), the Chinese Alisols ($P < 0.05$), and in tendency also for the Fluvisol (significance not tested due to n=1 profile pair), whereas

no such effects were found for the Javanese Alisols and the Vertisols (Fig.II 1). Hence, the N stocks of the topsoils were significantly different between soils developed on contrasting parent materials ($p<0.01$), except between Vertisols and Chinese Alisols ($p=0.9$).

Paddy management did not affect N stocks in the subsoils ($p>0.05$). The N stocks of the subsoils also showed minor differences between the soil groups. Only the subsoils of the Javanese Alisols were significant different from all other subsoils ($p<0.05$). Total stocks of N were largest for the volcanic soils (Andosols and Javanese Alisols) with high oxide and reactive minerals stocks (sum of dithionite-extractable Fe_D, Mn_D, and Al_D; Fig. S1, Supplementary Materials) and smallest for oxide-poor soils. Overall, we found a weak correlation between N stocks to Al_D and Fe_D stocks ($R^2=0.35$-0.45, $p<0.05$; Fig.VI 2 in Appendix A). No clear relations of N to other soil properties such as texture, CEC, or pH were found.

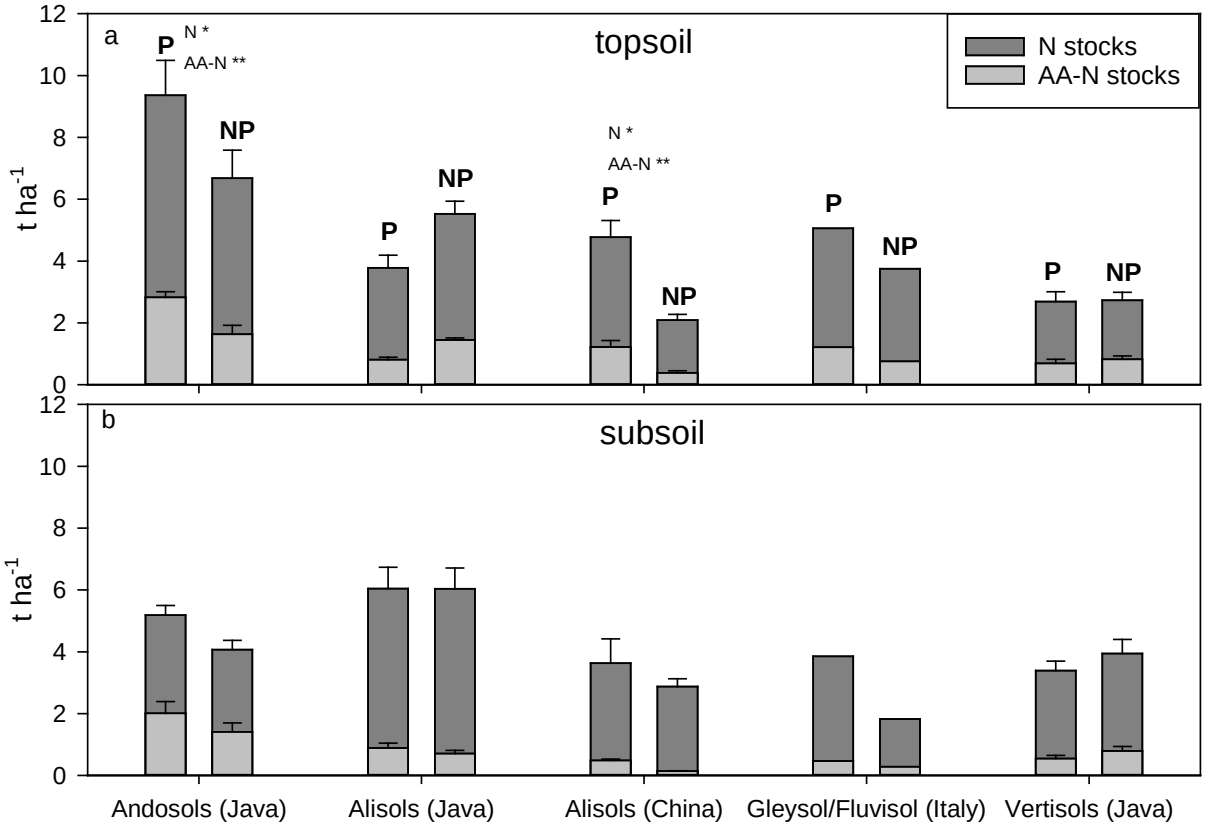


Fig.II 1: (a,b) Soil nitrogen (N) including amino acid-N (AA-N) stocks in paddy (P) and non-paddy (NP) (a) top- and (b) subsoils of five major reference soil groups (3 field replicates, except for Italian site); the asterisks * and ** indicate significant difference between paddy and non-paddy management at $p<0.05$, and $p<0.01$ level of probability, respectively. Additional effects on N accumulation may result from Mimosa and glover cropping in NP-Alisols (Java) and P-Alisols (China), respectively.

Amino acid stocks

In the studied soil horizons, 7-40% of N was bound in amino acids. Especially in topsoils and subsoils of the Chinese Alisols, amino acid contents were low (Fig.II 1), and so were the contributions of amino acids to total N (Fig.II 2).

When comparing all paddy and non-paddy managed soil profiles, there were no significant differences in the proportion of AA-N to N among sites, except for the Chinese Alisols ($p<0.05$). The top- and subsoils of the paddy managed Chinese Alisols contained significantly larger AA-N stocks than their non-paddy counterparts ($p<0.05$).

Between the major reference soil groups, the topsoil AA-N stocks were significantly different ($p<0.01$), except between Vertisols and Chinese Alisols ($p= 0.5$). Hence, the AA-N stocks were rather related to site than to differences in paddy/non-paddy management. In Andosol top- and subsoils, N comprised 30 - 40% in amino acids, a portion, which was not reached by the other four soil groups (Fig.II 2). In general, the soils with the largest N stocks also tended to have the largest AA-N stocks (Fig.II 1, Fig.II 2).

We assumed that the soil mineral assembly controlled the degree of microbial N sequestration. However, we did not find any correlations between AA-N stocks with soil variables such as particle size distribution, pH, DCB-extractable Fe_D, Al_D, and Mn_D stocks (Fig.VI 2, in Appendix A) or CEC (linear regression $R^2=0.1$-0.3, $p<0.05$). Also, we found no correlation of AA-N stocks to oxalate-extractable Fe and Al contents (data from Winkler et al., 2016). Besides, when using a multiple linear regression to determine the influence of two or more independent variables on the stocks of AA-N or their contributions to soil N, we did not obtain a coefficient of variation exceeding 0.2-0.3 (e.g., Fig.VI 2 in Appendix A). Therefore, we have to refrain from relating overall N sequestration in amino acids to single soil group specific properties.

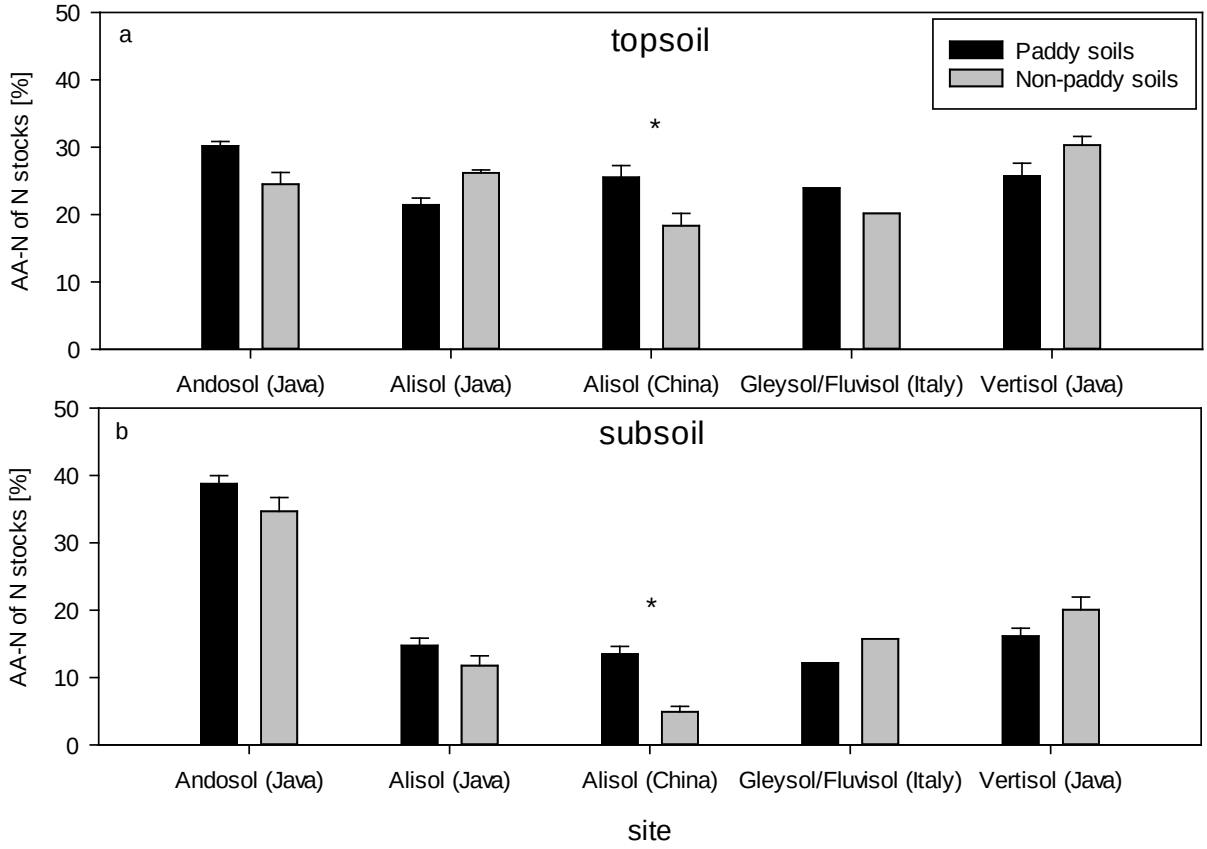


Fig.II 2: Percentage of N in amino acids (AA- N) of total N stocks in paddy and non-paddy (a) top- and (b) subsoils of five major reference soil groups (3 field replicates, except for Italian site). The asterisk * indicates significant difference between paddy and non-paddy managed top- and subsoils of Chinese Alisols at $p<0.05$ level of probability.

Amino acid composition

Alanine, methionine, leucine, isoleucine and proline are nonpolar amino acids. Tyrosine, threonine, glutamine and glycine belong to the class of neutral amino acids, and glutamic acid and aspartic acid to the acidic ones. Lysine is an alkaline amino acid. When grouping the amino acids according to these functional groups, we found that nonpolar amino acids constituted the largest portion of detected amino acids, followed by acidic, neutral and alkaline ones (Fig.II 3). This pattern was observed for all studied soils, with no significant differences between paddy and non-paddy soils and between the different soil depth intervals. Only when comparing the amino acid composition among the soil groups, our data pointed to lower proportions of non-polar and elevated proportions of acidic amino acids in the Andosols ($P < 0.05$;Fig.II 3). The amino acid composition in the other four soil groups remained fairly similar.

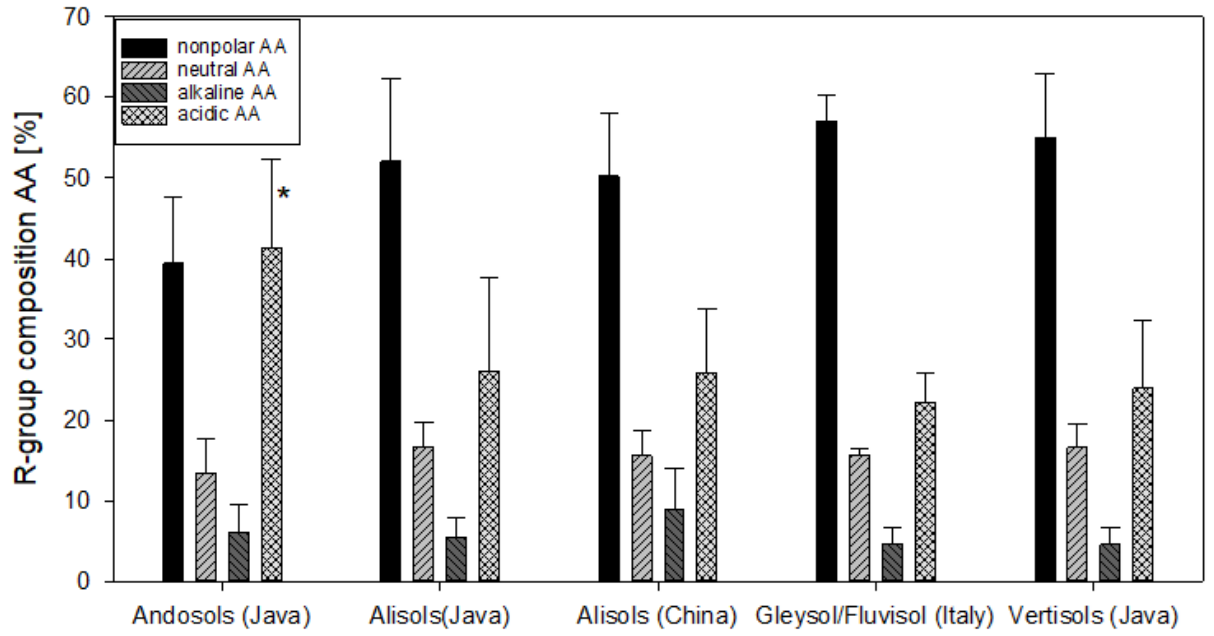


Fig.II 3: Charge distribution (R-group composition) of amino acids of five major reference soil groups (summarized for paddy and non-paddy managed soils; 6 field replicates); the asterisks * indicate significant difference between soil groups regarding acidic amino acids at $p<0.05$ level of probability.

D-/L-ratios of amino acids

The D-/L-ratios ranged from 0.09 to 0.41 for alanine, from 0.07-0.20 for glutamic acid, from 0.05 to 0.91 for aspartic acid, and from 0.01 and 0.11 for lysine (Tab.VI 1 in Appendix A). Hence, there was a considerable variation in amino acid chirality. The D proportions of other amino acids were not evaluated in detail, as they are not specific for microbial origin or ageing processes (Amelung et al., 2008). Across all sites, the D-/L- ratios increased with increasing depth within the soil profiles. The most distinct depth gradients were observed for Andosols while those in the Vertisols were rather weak (Fig.II 4; for lysine data see Tab.VI 1 in Appendix A). Significant differences in the D-/L- ratios between paddy and non-paddy managed soils were not evident ($P > 0.05$). For better comparability between the soil groups (independent from management), we averaged individual D-/L- ratios over the depth profiles, as shown in Figure II 5. This revealed largest D-/L- ratios of alanine and aspartic acid in the Andosols, followed by the two Alisols sites (Indonesia and China), the Fluvisol and the Vertisols. The D-/L- ratios alanine of the top- and subsoils of the Andosols and Javanese Alisols were significantly higher compared to the Vertisols, Chinese Alisols and the Gleysol/Fluvisol pair ($p<0.01$, Fig.II 5a). For the D-/L- ratios of aspartic acid only the Andosols significantly differed from the other soil groups ($p<0.001$ in top- and subsoils, Fig.II 5c). This suggests that higher D-enantiomer contributions of alanine occurred in oxide-rich

soil. We also found correlations between of D-/L- ratios of alanine to Fe_D and Al_D stocks (R^2 = 0.45-0.53, respectively; $p < 0.05$, Fig.VI 2 in Appendix A). The D-/L- ratios of lysine did not follow this trend. They were largest in the Fluvisol, followed by Alisols. These differences were caused by elevated D-/L- ratios of lysine in the respective subsoils (Tab.VI 1 in Appendix A).

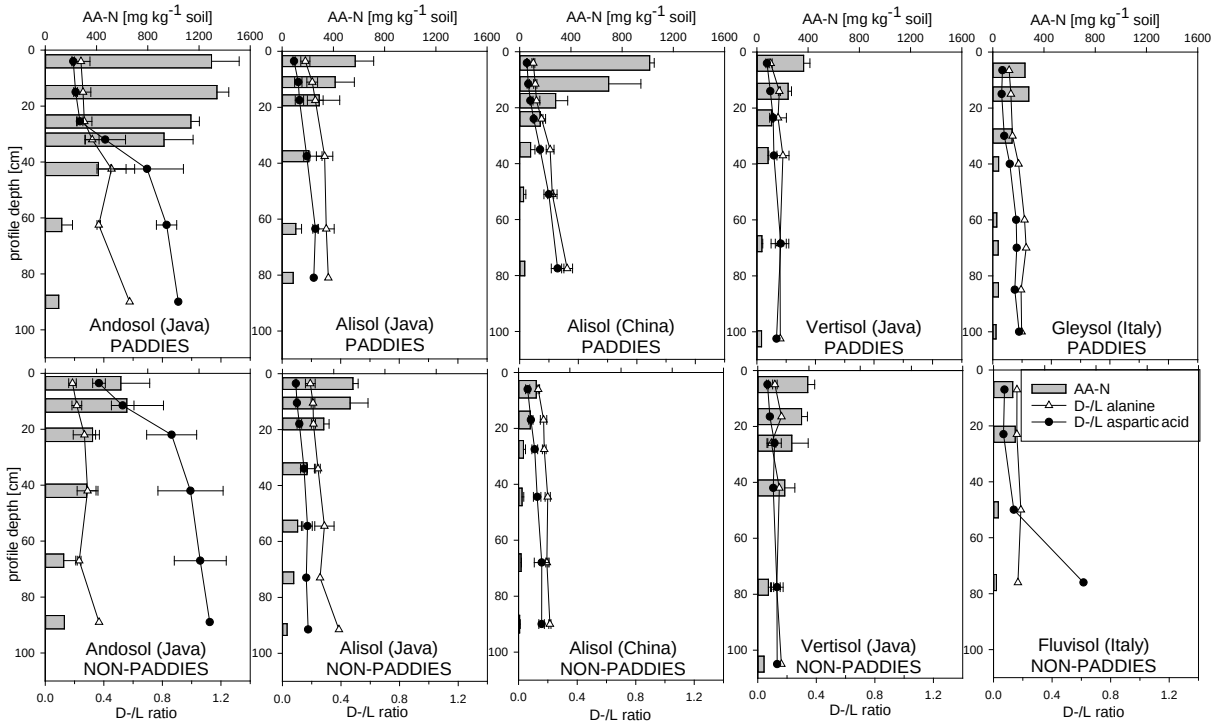


Fig.II 4: Amino-acid-N contents (mg kg^{-1} soil) and D/L-ratios of alanine and aspartic acid in soil depth profiles.

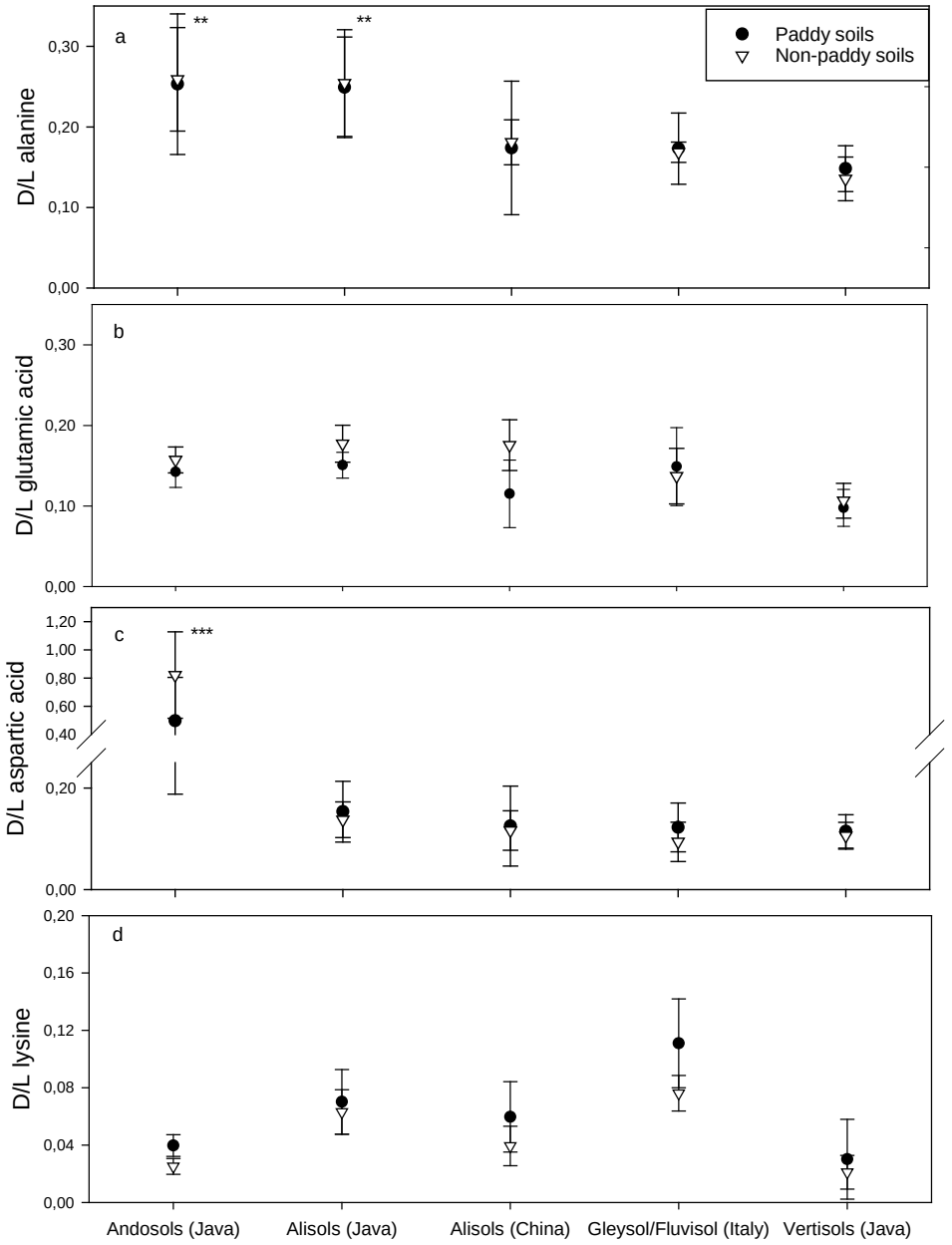


Fig.II 5: (a-d) D/L ratios of a) alanine, b) glutamic acid, c) aspartic acid and d) lysine in paddy and non-paddy soils of five major reference soil groups (averaged for 100 cm soil depth; 3 field replicates); the asterisks ** and *** indicate significant difference between soil groups at $p<0.01$, and $p<0.001$ level of probability, respectively.

Amino acid signatures after DCB treatment

Treating the soils with DCB resulted in a loss of 0-155 mg AA-N kg^{-1} relative to the original bulk soil contents (Fig.II 6). The extent of AA-N loss was not related to paddy management; samples in a given major reference soil group lost the same proportions of AA-N irrespectively of the management. Across the different soils, the Javanese Alisols and the Andosols exhibited the largest Fe_D contents and also showed the largest losses of AA-N after DCB treatment. Nevertheless, the overall correlation of DCB-induced AA-N losses to Fe_D

contents was not significant (R^2= 0.37; p= 0.1) (Fig.II 6). We did find a significant correlation between the decrease in AA-N after DCB treatment and DCB-extractable Al contents (R^2=0.8, p<0.001; Fig.II 6). The losses of AA-N were smallest for the Al_D-poor Fluvisol and Vertisols, and largest for the Al_D-rich Andosols.

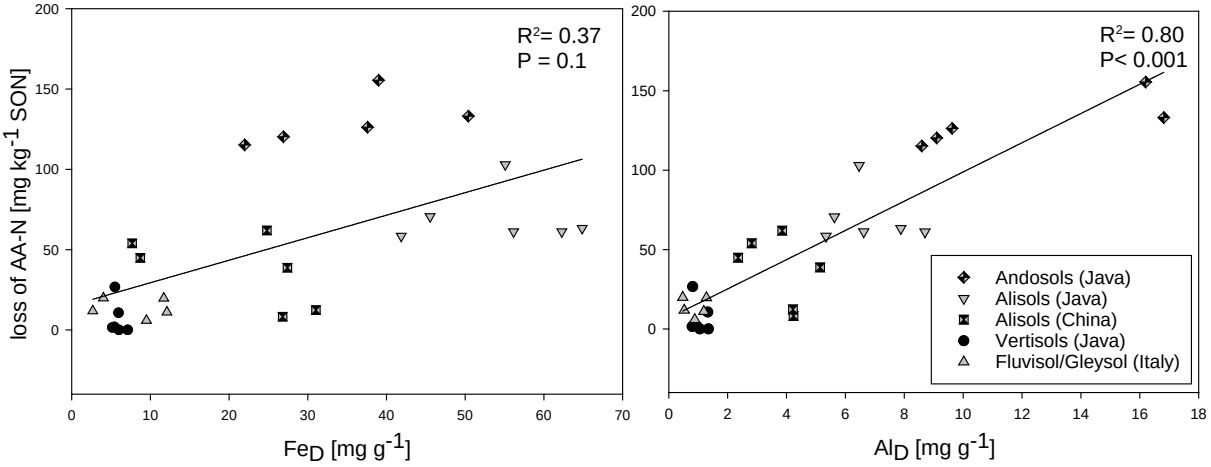


Fig.II 6: Changes in the contributions of N in amino acids (AA-N) to total N versus DCB extractable Fe (Fe_D) and Al (Al_D); top-and subsoil samples, three paddy and three non-paddy sites per reference soil group are shown.

4 Discussion

Paddy management and soil organic nitrogen and amino acid-N accumulation

Paddy soil management has been shown to promote SON accumulation due to anaerobic conditions and related slow organic matter decomposition, leading to almost twice as much N in soils under paddy compared with those under non-paddy management (Kögel-Knabner et al., 2010, Roth et al., 2011; Jiang et al., 2013). However, Tong et al. (2009) noted that SON accrual may also depend on other site conditions that may be as relevant as flooding for maintaining high N contents. This agrees with observations by Winkler et al. (2016) and Lehndorff et al. (2016) who did not find a preferential enrichment of organic C and black C accumulation in some major reference soils upon paddy management. The authors also noted that rather site-specific changes in input and soil properties affected the C contents in soil. We made similar findings. N and amino acid accumulation did not only occur in paddy soils but, at least partly, to a similar extent in adjacent non-paddy soils. Obviously, there are other factors than paddy management that may account for a preferential sequestration of N and amino acids in the studied soils. The lack in differences in N stocks between paddy and non-paddy soils may either be caused by elevated SON levels in non-paddy soils, e.g., due to the

cropping of N-fixing plants, or due to inefficient SON storage in paddy soils, e.g., by lacking impermeability of the plough pan of the paddy soil (Kölbl et al., 2014).

In our study, paddy and non-paddy soils were cropped with mimosas, sugar cane, and clover. These N-fixing plants are propagated in many tropical lowland areas because they enrich SON (van Kessel et al., 1994; Giller, 2001). Mimosa trees (*Leucaena leucocephala*) were grown on non-paddy Alisols in Java, which likely even led to significantly larger AA-N stocks in the non-paddy topsoils than in those of the paddy managed counterparts ($p<0.05$;Tab.II 2, Fig.II 1). Clover was cropped in rotation with paddy rice in the Chinese Alisols. In these paddy soils, the AA-N stocks were significantly elevated relative to the respective non-paddy soils ($p<0.001$; Fig.II 1). The non-paddy Vertisols were cropped with sugar cane, which also promotes N accumulation via biological N fixation (Lima et al., 1987, Boddey et al., 1995). Nevertheless, N and AA-N stocks in Vertisols were lowest for all soils under study and no differences between the managements were observed (Fig.II 1, $p= 0.2$). This is surprising since clay is supposed to stabilize organic matter in soil (Ladd et al., 1996). Likely, the N was transformed prior to microbial sequestration and stabilization in microbial residues, which may, e.g., occur during dry seasons followed by high denitrification rates and leaching during rainy seasons. This led to enhanced N losses in these self-mulching, clay-rich soils (Patra and Rego, 1997). A lack in long-term sequestration of N is also supported by relatively large C/N ratios in the Vertisols (~ 13 compared to ~ 10 for the other soils, data not shown). The organic matter accumulation in paddy soils was also related to formation of a dense plough pan, which limits translocation of organic compounds into the subsoils (Kögel-Knabner et al., 2010; Kölbl et al., 2014). For the Italian paddy soil (Gleysol), however, the coarse texture (50% sand-sized particles) inhibits plough pan formation and allows for lateral organic matter translocation (Said-Pullicino et al., 2015). A similar process may have taken place in the Javanese Alisols under study (Alisols had 15-50% sand-sized particles), further masking the effect of organic matter accumulation in topsoils on N stocks in paddy-cropped Javanese Alisols. In this regard, the coarse texture may have contributed to inefficient SON enrichment in the Gleysols and Javanese Alisols under paddy management, which therefore also lacked steep depth gradients in amino acid depth profiles (Fig.II 4).

Andosols are known to exhibit high stability of microaggregates, low bulk density (Wada, 1985) and large portions of reactive allophane minerals (Mikutta et al., 2010) which all should facilitate SON accumulation. Inubushi et. al. (2005) reported larger contributions of microbial biomass-N to total N in soil for Andosols. Other authors supported their C-turnover models by implementing different microbial turnover efficiency for paddy and non-paddy soils

(Shirato et al., 2005). In our study, the Andosols had the most significant positive effect on SON accumulation and this was even higher under paddy management (Fig.II 1). Soil-specific biogeochemical processes may thus also affect the extent of SON and protein-N accrual.

Soil-related ageing of amino acid N

Besides the option to accumulate total peptide-bound N in paddy soils by decelerated degradation of organic matter, soil specific properties might lead to preferential accumulation of amino acids, e.g. due to sorption to reactive soil phases and/or co-precipitation with pedogenic oxides (Miltner and Zech, 1999; Pan et al., 2004). If sorption plays a major role, it has been suggested that amino acids with certain charge distribution accumulate in preference over other amino acids (Mikutta et al., 2010). In this study, only Andosols (paddy and non-paddy soils) were slightly enriched in acidic amino acids (Fig.II 3), as may be explained by preferential sorption of these amino acids to reactive allophane and oxide phases (Mikutta et al., 2010). Yet, we did not find systematic differences in amino acid composition between paddy and non-paddy management and among the other reference soil groups. Similarly, also other authors failed to find close correlations of amino acid charges to basic soil properties such as pH, base saturation, CEC, and clay content (Friedel et al., 2002; Amelung et al., 2006). We hydrolyzed proteins, hence amino acids discussed here originally did not exist in soil in free forms but in protein complexes, which likely contained a range of different amino acids rather than specifically charged ones. Hence, we concur with Amelung (2003) that the dominant control on amino acid composition in soil is likely driven by microorganisms: once they have access to specific SON sources, they consume them completely rather than leaving parts of the protein complex or certain peptide-bound amino acids behind. When amino acids originate from bacterial cells, the D-content of alanine and glutamic acid increases, because these amino acids are indispensable parts of peptidoglycan cell walls (Nagata et al., 1998; Pelz et al., 1998; Amelung et al., 2008). When soil proteins are stored for prolonged periods of time, their amino acids may age, i.e., enzyme-catalyzed or abiotic racemization reactions result in elevated D-contents of aspartic acid and lysine (Kvenvolden et al., 1970; Amelung, 2003). Here, we observed that the portion of D-enantiomers of all these amino acids generally increased with increasing soil depth, reflecting increased input by leaching, transformation and ageing of the respective N forms in subsoils (Fig.II 4). The preferential storage of D-amino acids in the subsoil may be accompanied by chemical bonds to or general other stabilizing associations with soil minerals (Mikutta et al., 2010). In line with that, we

observed an increasing portion of unidentified N with increasing soil depth (Fig.II 4), while C/N ratios remained rather uniform throughout the depth profiles except for the Chinese Alisols were C/N ratios dropped to ~ 5 in the subsoil indicating accumulation of strongly degraded organic matter in the subsoil (data not shown). The time-scales of SON transformation processes are still unknown. Radiocarbon ages of subsoil organic matter in a Chinese paddy soil chronosequence approached a few thousands of years (Wissing et al., 2011; Bräuer et al., 2013), therewith exceeding by far the time scale of paddy management at the sites under study (>100 years). Likely, a majority of SOM in the subsoil was possibly already just too old to be detectably affected by >100 years paddy management, which, in addition, mainly affects the SOM of the topsoils (Kögel-Knabner et al., 2010; Wissing et al., 2011). Thus, it seems likely that also the formation of the D-amino acid enantiomers in the subsoils extended far beyond the time of paddy management. Intriguingly, the D-/L- ratios of the amino acids differed among soil groups. Highest D-/L- ratios of alanine were found for the Andosols and Javanese Alisols, which also had the largest amounts of soil N. As D-alanine is produced by bacteria (Pelz et al., 1998; Amelung et al., 2008), the N accrual in these soils likely was accompanied by microbial transformation of N and related stabilization processes. The close correlation of Al_D and D-alanine may suggest that the sequestration of bacteria-derived N may be related to Al-enriched oxide phases (Fig.VI 2 in Appendix A). The correlation between the loss of amino acids after DCB extraction and the contents of indicators of oxides is in support of this hypothesis. Yet, the amino acid loss after DCB treatment was independent from management (Fig.II 6), maybe, because this treatment also affected oxides and amino acids older than those formed during the last century of paddy soil use.

The release of Al by DCB is chemically difficult to explain, because Al solubility should be independent from pH-buffered redox treatments. We speculate that there was an atomic substitution of Fe by Al in more crystalline Fe oxide phases, such as in goethite. Aluminum substitution in goethites is well-known and can reach up to 33 Mol% (e.g., Fitzpatrick and Schwertmann, 1982). Close correlations between the stocks of Al_D with the larger stocks of Fe_D support this hypothesis (Fig.VI 3 in Appendix A). The Al substitution in goethites results in increased specific surface area and increased anion (phosphate) sorption per surface (Ainsworth et al., 1985; Borggaard et al., 1990). The loss of Fe by DCB treatment may simply cause co-loss of Al, with soils being most rich in Fe oxides such as the Javanese Alisols having also the highest possibility to contain DCB-extractable Al. The Javanese Alisols also showed a pronounced accumulation of bacterial D-alanine (Fig.II 4, Fig.II 5).

5 Conclusion

Paddy management influences both peptide N accrual and related microbial N transformation. This study showed that these effects, however, are modulated by site-specific management and parent material, yet without significant impacts on the composition and chirality of amino acids. Microbially transformed amino acids were enriched in Andosols but hardly accumulated in Vertisols. As oxide removal went along with a loss of amino acids including those typical for peptidoglycan, we infer that oxidic mineral phases controlled long-term organic N cycles irrespective from current land-use. Elucidating the net rates of microbial N accrual, e.g., by using compound-specific stable isotope analyses and radiocarbon dating, might now warrant further attention.

III.

NITROGEN IMMOBILIZATION IN MICROBIAL RESIDUES OF PADDY SOILS DEVELOPED ON CONSTRATING PARENT MATERIAL

Modified on the basis of:

Nitrogen immobilization in microbial residues of paddy soils developed on contrasting parent material

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1 Introduction

In paddy soils, nitrogen-use efficiency is frequently smaller than 40%, inter alia due to denitrification processes and ammonia volatilization into the atmosphere (Cassman et al. 1996, Guo et al. 2017, Wang et al. 2018). Several studies showed that also microorganisms may contribute to the low N use efficiency in paddy soils by immobilizing N in their residues (Ding et al. 2011, Roth et al. 2011, Cucu et al. 2013, Said-Pullicino et al. 2014). This N sequestration process operates at a scale of decades (Roth et al. 2011). The analyses of amino sugars help to differentiate and quantify the amount of N that is bound in the residues of bacteria and fungi (Amelung et al. 1999, Liang et al. 2007). Glucosamine, for instance, mostly originates from fungal chitin, while muramic acid uniquely derives from bacteria (Parsons 1981, Glaser et al. 2004). Whether the sequestration of N in paddy soils correlates to microbial immobilization of N and if fungi or bacteria systematically dominate in this periodically flooded soil environment is not yet clear.

Soil properties may influence microbial N sequestration in paddy soils. In particular it has been assumed that frequent submergence promotes the stabilization of organic matter as occlusion in pedogenic oxides formed after repeated wetting and drying cycles (Olk et al. 1996, Kögel-Knabner et al. 2008, Said-Pullicino et al. 2014, Chen et al. 2014). Accordingly, for peptide-bound amino acids a preferential sequestration in oxide-rich Andosols and Alisols was found (same sample set; see Houtermans et al. 2017). The current study was performed to clarify whether and to which degree oxides also promote the accrual of microbial residues in paddy soils relative to their non-paddy counterparts.

Any microbial transformation of soil organic nitrogen (SON) results in a discrimination of the ^{15}N versus the ^{14}N isotope, because microbes prefer the lighter ^{14}N during metabolic reactions, thus leaving the heavier ^{15}N isotopes behind. As a result, the soil $\delta^{15}N$ values are usually larger than that of plant and fresh litter inputs, and they increase with increasing degree of soil N turnover (Dijkstra et al. 2006). Therefore, we use the $\delta^{15}N$ values of bulk soil to indicate differences in microbial N turnover and overall N use efficiency as affected by paddy management or major soil groups.

As along with soil mineral assemblage also microbial community structure changes (Steinbach et al. 2015, Mejia et al. 2016), we assume that the contribution of fungal and bacterial residues to total N immobilization should also be affected by the mineral assembly in paddy soils of different parent material. Fungi, for instance, frequently dominate in acidic soils with low availability of nutrients, but with elevated contents of recalcitrant organic matter, usually with wide in C/N ratio (Holland und Coleman 1987, Blagodatskaya und

Anderson 1998). Fungal hyphae grow slowly, but they are more resistant against further microbial decomposition than bacterial polymers (Webley und Jones 1971). In nutrient-rich soils, in contrast, bacterial communities are abundant because of their ability to grow rapidly and, therewith, to take advantage of elevated nutrient supply. Bacteria thus also respond fairly quickly to environmental changes (Ingwersen et al. 2008, Hayat et al. 2010). As paddy soils may receive high fertilizer applications (Kögel-Knabner et al. 2010), it has been assumed seemed reasonable to hypothesize that paddy management favors bacterial growth rather than that of fungi (Dong et al. 2014). For instance, Dong et al. (2014) found that the organic manuring common in paddy management promoted the activity of bacteria. Also Roth et al. (2011) determined an elevated bacterial N sequestration in paddy topsoils compared to non-paddy soils. Nothing is yet known, however, on how different mineral assemblages alter the ratio of bacterial to fungal residues in paddy soils.

Here, we hypothesized that relative to upland soils mainly bacteria contribute to the sequestration of N in microbial residues and may reflect anthropogenic land-uses, whereas fungal residues will reflect differences in the role of parent material on microbial N sequestration and may reflect long-term soil development. Consequently, we sampled triplicates of five pairs of paddy and non-paddy soils formed on different parent material (Andosols, Alisols, Vertisols, and a Gleysol/Fluvisol pair). We determined the stocks of soil organic carbon (OC), total N (N_t), amino sugar-N (AZ-N), calculated microbial residue stocks and composition (bacterial and fungal), and assessed the $\delta^{15}N$ isotope composition of the bulk soils. We then related these data to information about substrate and pedogenic processes, i.e. grain size distribution and iron oxide crystallinity (data from Winkler et al. 2016).

2 Materials and methods

Sampling sites

For detailed information see chapter II 2 Material and methods. All information about individual site management, such as crop rotation, yield, fertilizer application and herbicides were gained by interviewing the local famers (for a summary see Tab.VII 1 in Appendix B).

Andosols, Alisols and Vertisols were located in Java (Indonesia) and developed from similar volcanic substrate and under similar climatic conditions (tropical monsoon climate, 21-27°C mean annual temperature, precipitation varies from 1900 – 6700 mm a^{-1}). The Andosols were sampled on a terraced slope near the city Sukabumi and were under rainfed arable land use. The paddy fields were cropped two times with rice and one time with Chinese cabbage per year. The tillage happened by hand tractors. The farmers applied Urea, NPK, SP-36

(superphosphate, 36%) and partly manure. The famers estimated their yield to 5.000 to 6.000 kg rice/ha/year. The non-paddy fields were cropped with cassava (6 month rotation) and maize (yearly rotation). The tillage was made with a hoe. Manure, urea and NPK were applied as fertilizer.

The Vertisols were located in a river valley near Ngawi. The paddy fields were cropped with two times of rice and one time of tobacco per year. The rice farmers used 50 kg/ha NPK, 50 kg/ha ammonium sulfate, 25 kg/ha urea, 25 kg/ha TSP (triple superphosphate, 46%) and 25 kg/ha SP-36 per year as fertilizer. The non-paddy fields were cropped with sugar cane. The sugar cane farmer used fertilizer in form of green manuring 1000 kg/ha, ammonium sulfate 200 kg/ha, NPK 600 kg/ha and year. At the Vertisol site the famers used groundwater for irrigation and the water table was <2 m beneath the soil surface.

The Javanese Alisols were located in a small plain near Jasinga. The paddy fields were cropped with two times of rice and one time of maize per year. The tillage was made with buffalos. The paddy fields were fertilized with 300 kg/ha urea, 200 kg/ha NPK, SP-36 and manure. The farmers estimated their yield to 3000 kg rice/ha/year. The non-paddy fields were cropped with cassavas, bananas and scattered leguminous trees (boundary planting). The farmers used hoes for tillage and manure for fertilization.

The Chinese Alisols developed from red sandstone near Jiangxi (Red Soil Ecological Experiment Station, China). The climate is monsoon-influenced humid tropical with mean annual temperature of 18°C and mean annual precipitation of 1600-1700 mm. The paddy fields were at the bottom of a terraced slope and the non-paddy fields were located at the upper slope. The paddy fields were cropped with two periods of rice per year and in the following year with clover. The non-paddy fields were cropped with peanuts, sesame and sweet potatoes. We had no information about fertilization.

The Gleysol and Fluvisol pair was located in the plain of the Po River at Zeme, Italy (Rice Research Center of Ente Nazionale Risi). The climate is temperate with mean annual temperature of 13°C and mean annual precipitation of 700-1000 mm. The Gleysol was cropped with one period of rice per year without puddling but with low ploughing. The Fluvisol was cropped with one period of maize per year. Both fields were fallow during winter. For detailed soil horizon description see Winkler et al. (2016).

C, N and $\delta^{15}N$ analyses

The analysis of total C and N was carried out in duplicate with an elemental analyzer (Vario MICRO cube, Elementar Analysensysteme GmbH, Hanau, Germany). We determined the

$\delta^{15}N$ isotope ratio in duplicate by elemental analysis - isotope ratio mass spectrometry (EA-IRMS; Flash EA 1112 coupled to DeltaV Advantage, ThermoFisher GmbH, Schwerte, Germany). The isotope ratio was expressed as $\delta^{15}N$ in ‰ using $\delta^{15}N$ of atmospheric air as a reference. Analytical drift was corrected by external standards acetanilide (1.18 ‰ $\delta^{15}N$) and ammonium sulfate (20.3 ‰ $\delta^{15}N$).

Analyses of amino sugars

We analyzed four amino sugars: glucosamine (Glu), galactosamine (Gal), mannosamine (Man) and muramic acid (MurA). Amino sugars were detected according to Amelung and Zhang (1996). Samples were hydrolyzed with 6 M hydrochloric acid and recovery standard (myo-inositol) was added. The hydrolysate was filtered and dried. The purification was conducted in two steps, first solution was adjusted to pH 6.6-6.8 and centrifuged, and then the freeze-dried supernatant was washed with methanol. Samples were derivatized to aldononitrile derivates using hydroxylamine hydrochloride and dimethylaminopyridine, followed by acetic anhydride addition. After derivatization the quantification standard was added (β-endosulfan).

The four amino sugar derivatives were measured with a gas chromatograph - flame ionization detector (GC – FID) (Agilent 6890, Agilent GmbH, Böblingen, Germany) using a capillary column (Optima-5 MS; 30 m, I.D. 0.25 mm; Macherey Nagel). Detailed method description and temperature program see Zhang und Amelung (1996). The recovery of the first internal standard myo-inositol exceeded 70 % in all runs.

Calculations and statistics

Appuhn und Joergensen (2006) measured the amino sugar content of bacterial tissue from rhizosphere soil and root material and compared the data with total microbial biomass based on fumigation extraction and ergosterol data. On the basis of these experiments they assessed a conversion value of 45. Hence, we used amino sugars as biomarkers for soil microbial residues. To estimate bacterial residues we used the muramic acid content and multiplied it with 45 (Appuhn und Joergensen 2006).

Fungal residue mass was calculated according to Engelking et al. (2007) and Appuhn und Joergensen (2006) (see equ. 1).

(1) Fungal residue C [mg g^{-1}] = (1x [mg g^{-1}] glucosamine – 2x [mg g^{-1}] muramic acid) x 179.2 x 9

Engelking et al. (2007) showed that muramic acid and glucosamine occur at a 1 to 2 molar ratio in bacterial cell walls. Appuhn und Joergensen (2006) thus generated a conversion value of 9 for fungal residues. Total microbial residues were the sum of bacterial and fungal residues.

To compare different soils we calculated bacterial and fungal residues stocks (based on bulk density and soil depth).

SigmaPlot 13.0 for Windows (Systat Software) and STATISTIKA 8.0 (Stat-Soft, Inc.) were used for statistics. Differences in total N stocks, AZ-N stocks and microbial residue stocks of the top- and subsoils were tested between soils (ANOVA) and for the combined effect of paddy and non-paddy management of the different soil groups (MANOVA). Linear and multiple regression was tested for correlations between DCB Fe-, Al and Mn stocks, pH, CEC, particle size distribution with amino sugar-N -, microbial residue stocks and $\delta^{15}N$ values.

The tests were run at a confidence level of 95 %. The results were considered to be significant at $p < 0.05$ and highly significant at $p < 0.01$.

3 Results

We assumed that the total N amount of N bound in amino sugars (amino sugar-N) showed significant differences between paddy and non-paddy managed soils. Surprisingly, this was not the case: instead, the stocks of total N (N_t) and amino sugar-N was similar for soils under paddy and non-paddy use, except for Andosols, which showed significantly larger N_t and amino sugar-N stocks under paddy management than their non-paddy counterparts (Fig.III 2). However, we found that the stocks of N_t and amino sugar-N were different among the five soil groups.

We further assumed that the portion of bacterial to fungal residues would be larger in paddy soils than in non-paddy soils (Roth et al. 2011). We thus calculated fungal and bacterial residue stocks. Also here, contrary to our expectations, we did not find that paddy management consistently enhanced bacterial residue stocks. Only the Andosol derived paddies showed higher portions of bacterial residues than their non-paddy counterparts, and so did the Chinese Alisols. Apparently, the impact of paddy-management on specific microbial residue accrual was soil specific.

Across all soils and management types, the stocks of soil organic carbon (SOC) correlated with those of total N (Fig.III 1 a, $R^2= 0.96$, $p< 0.0001$, all top- and subsoils). Also, the stocks of Nt correlated with those of microbial residues (Fig.III 1b, $R^2= 0.68$, $p<0.0001$). In addition,

we found that presence of iron and aluminum oxides (dithionite extractable oxides, data from Winkler et al. (2016) correlated positively with the stocks of microbial residues (R^2= 0.51, p<0.001). Other soil properties like pH, CEC or texture did not show any significant correlation with SOC, N, amino sugar-N, or microbial residue stocks.

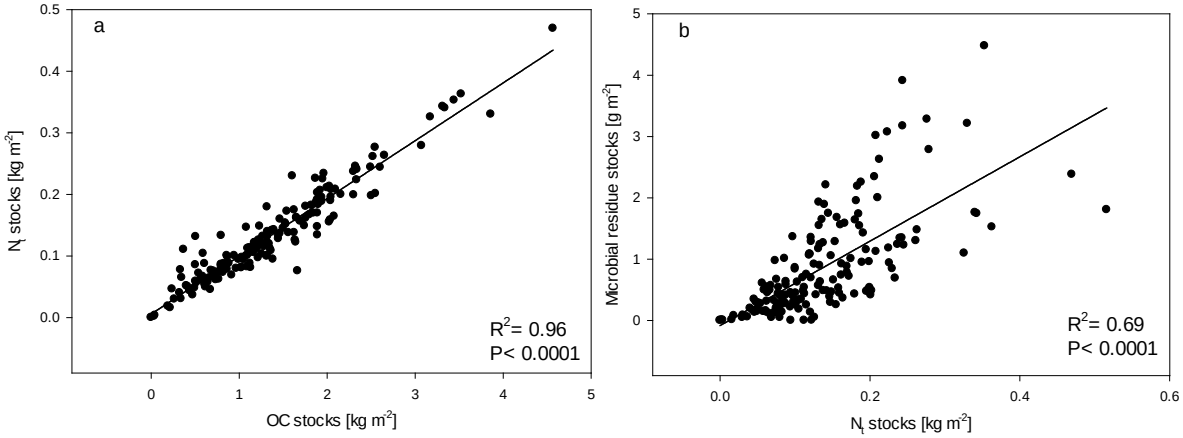

Fig.III 1: a) Correlation of organic carbon (OC) and nitrogen(N) stocks across all paddy and non-paddy top- and subsoils under study; b) correlation of the stocks of total nitrogen(Nt) with those of microbial residues (calculated from amino sugar analysis) across all paddy and non-paddy top- and subsoils under study.

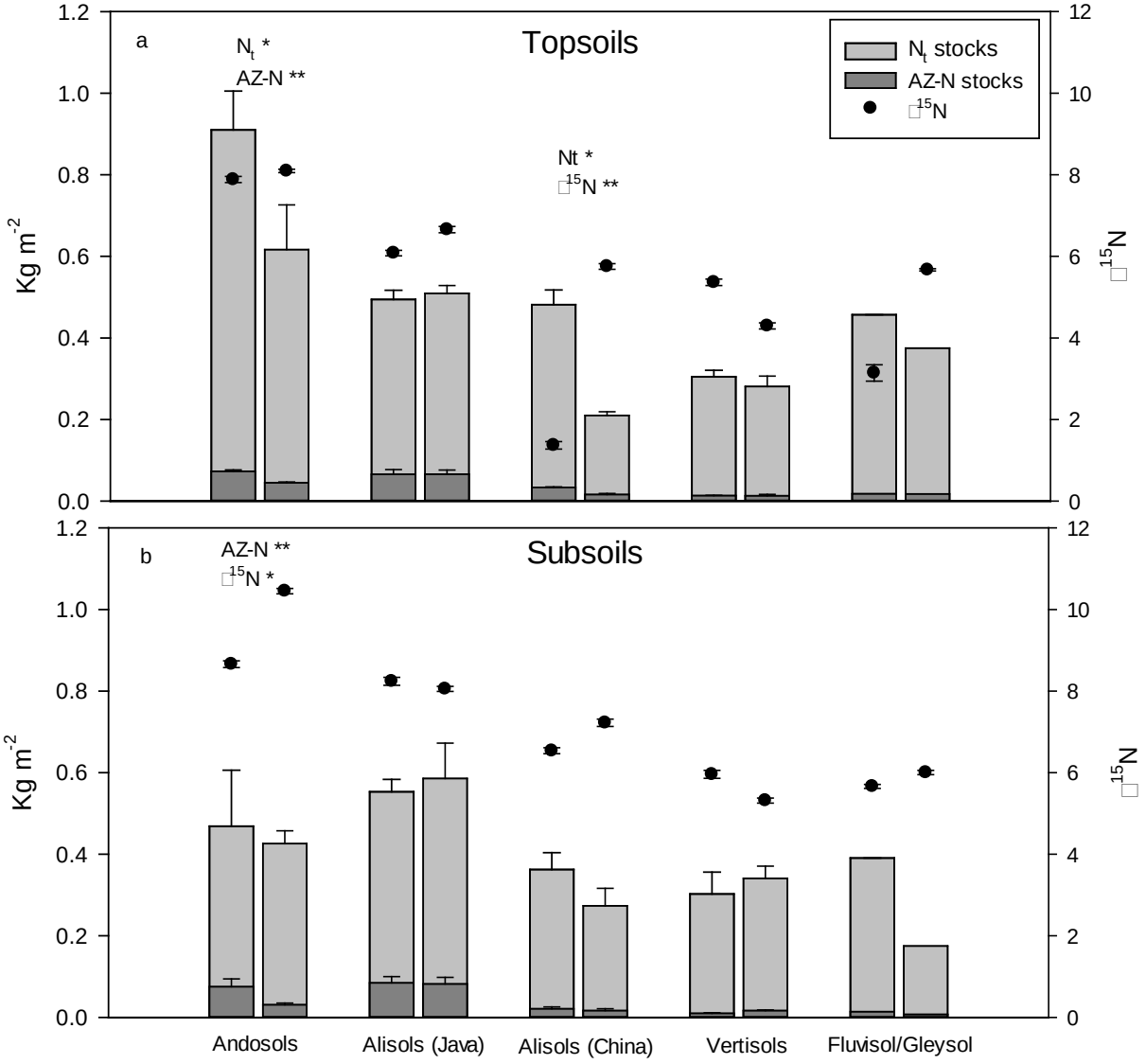

Fig.III 2: (a,b) Soil nitrogen (N) including amino sugar-N (AZ-N) stocks, and $\delta^{15}N$ in paddy (P) and non-paddy (NP) (a) top- and (b) subsoils of five major reference soil groups (3 field replicates, except for Italian site); the asterisks * and ** indicate significant difference between paddy and non-paddy management at $p<0.05$, and $p<0.01$ level of probability, respectively. Additional effects on N accumulation may result from Mimosa and clover cropping in NP-Alisols (Java) and P-Alisols (China), respectively.

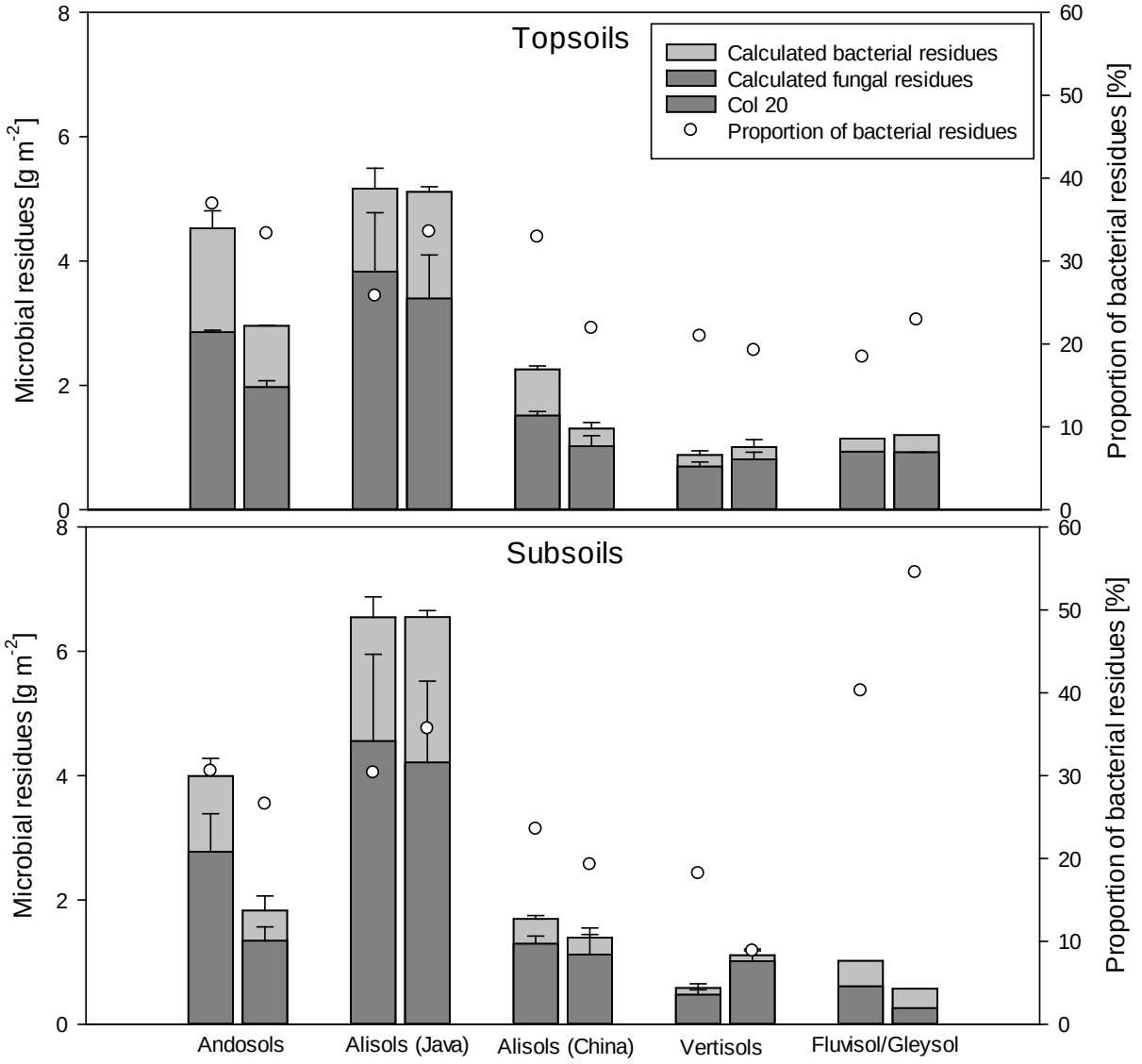

Fig.III 3: (a,b) Calculated microbial residue (mic) stocks including bacterial (bac) and fungal residue (fun) stocks (g m^{-2}), and proportion of bacterial residue to microbial residue stocks (%) in paddy (P) and non-paddy (NP) (a) top- and (b) subsoils of five major reference soil groups (3 field replicates, except for Italian site); the asterisks * and ** indicate significant difference between paddy and non-paddy management at $p<0.05$, and $p<0.01$ level of probability, respectively. Additional effects on N accumulation may result from Mimosa and clover cropping in NP-Alisols (Java) and P-Alisols (China), respectively.

Andosols

The Andosols showed the largest N_t stocks among all sites. The non-paddy arable topsoils showed significantly lower N_t stocks relative to their paddy managed topsoil counterparts (Fig.III 2a; $P < 0.01$). The N_t stocks of the Andosol subsoils did not differ between the two management practices (Fig.III 2b).

The N bound in amino sugars (amino sugar-N) comprised between 7 – 9% of N_t (Fig.III 2). The amino sugar –N stocks did not differ between the two management practices, neither for the top- nor for the subsoils (Fig.III 2a,b, $P > 0.05$); yet, microbial residue stocks exceeded

those of many other soils (Fig.III 3a). The $\delta^{15}N$ values were not affected by management. However, also the $\delta^{15}N$ values were larger for the Andosols (8 – 10‰; paddy and non-paddy top- and subsoils, Fig.III 2a, b) than for the other soil groups in our study.

Javanese Alisols

We did not observe any significant difference in N_t and amino sugar-N stocks of the Alisols collected in Java that relate to paddy and non-paddy management (Fig.III 2a, b). In the paddy and non-paddy topsoils, up to 12 – 14% of N_t were bound in amino sugars (Fig.1a). However, the composition of microbial residues changed with management: the contribution of bacterial residues to total microbial residues (32%) was significantly larger in the non-paddy topsoils than in those under paddy management (Fig.III 3a, $P < 0.05$).

In comparison with the other soil groups, the proportion of N_t, which was bound in amino sugars, even exceeded those of the Andosols (Fig.III 2). The respective microbial residues stocks were the largest found in this study. Besides, the Javanese Alisols had second highest $\delta^{15}N$ values among all studied soil groups (6 – 8‰; paddy and non-paddy top- and subsoils).

Chinese Alisols

In the topsoils of Chinese Alisols under paddy management, the N_t stocks were significantly larger than under non-paddy management (Fig.III 2a; $P < 0.05$). No significant differences between management practices were observed in the proportion of N_t bound in amino sugars (6% in the topsoils, Fig.III 2). However, both amount and proportion of bacterial residues were significantly larger in the paddy than in the non-paddy topsoils (Fig.III 3a, $P < 0.05$). In contrast to this, the $\delta^{15}N$ values of the paddy topsoils were significantly smaller than in the non-paddy ones (Fig.III 2, $P < 0.01$). The subsoils did not show any significant differences in N_t-, amino sugar-N stocks, microbial residues and $\delta^{15}N$ values between both management types.

Vertisols

For topsoils sampled from Vertisols we did not observe any significant differences in N_t-, amino sugar-N stocks and microbial residues that could be related to paddy and non-paddy management (Fig.III 2, Fig.III 3a,b). Only the subsoils of the Vertisols showed significantly larger contributions of bacterial residues to the total microbial residue pools when the soils were under paddy rather than under non-paddy use (Fig.III 3b, $p < 0.05$).

In comparison to the other soil groups, and despite having largest clay contents, the topsoils of the Vertisols showed the lowest N_t-, amino-sugar-N and microbial residue stocks among all sites under study (Fig.III 2, Fig.III 3a, b).

Gleysol/Fluvisol

The paddy topsoil at the Italian sampling site tended to have elevated N_t stocks compared with the non-paddy soil, but due to lacking independent site replicates this difference could not be tested statistically (Fig.III 2a). The amino sugar-N stocks did not indicate clear differences between management. However independent from paddy management, we did observed a higher portion of bacterial residues in the subsoils of the Gleysol/Fluvisol pair compared to the topsoils.

4 Discussion

Paddy rice management may accumulate organic N forms in soil (Roth et al. 2011). Suggested reasons are reduced decomposition of SOM under anaerobic conditions (Kögel-Knabner et al. 2010), abiotic reactions of NH_4^+ with phenols (Schmidt-Rohr et al. 2004), immobilization of N in microbial residues (Fuhrmann et al. 2018) and interactions of the latter or other N forms with the mineral phase (Miltner und Zech 1999, Mikutta et al. 2010, Mikutta und Kaiser 2011). With regard to SON, amino sugars can be bound to minerals by cation exchange reactions of their amino group or by ligand exchange with metal hyroxides by their carboxyl group (Cheshire et al. 2000). The latter differs between soil groups. In this regard, our study supported the hypotheses that the impact of paddy management on microbial N sequestration depends on the underlying parent material. Further, site-specific management practices contributed to differences in N sequestration.

Microbial N sequestration in soils formed on Fe and Al-rich substrates

Both, allophanes as well as Fe oxides, play a major role for SOM stabilization (Torn et al. 1997, Kögel-Knabner et al. 2008). Indeed, the Andosols exhibited largest N_t stocks as well as one of the largest microbial residue N stocks (Fig.III 2, Fig.III 3). In these latter soils, the volcanic parent material provides short range-ordered Fe oxides and allophane-imogolite-type phases that could support the sequestration of SOM (Winkler et al. 2016), and, thus, also of N_t. Moreover, also microbial residue N was enriched in Andosols though at similar magnitude as in the Javenese Alisols (Fig.III 2a, b). The latter are also rich in pedogenic oxides (Winkler et al. 2016). It seemed thus reasonable to conclude that pedogenic oxides play a major role for

N sequestration. And indeed, across all soils under study, the stocks of oxides (DCB extractable Fe, Al, Mn) showed a positive correlation to both the stocks of amino sugar-N ($p<0.01$) and, thus, of the total microbial residue pool ($R^2= 0.60$, Fig.III 4).

Amelung et al. (2001) reported that the addition of oxides to decaying litter retarded the synthesis of bacterial residues but enhanced the storage of N in fungal remains. The findings might imply a more important role of fungi for N sequestration in oxide-rich soils. We here also found a better correlation of pedogenic oxide contents with fungal residues ($R^2= 0.62$) than with bacterial ones ($R^2= 0.50$), despite bacterial residues showed significant contributions to total microbial residue N stocks in Andosols and Alisols (Fig.III 3a).

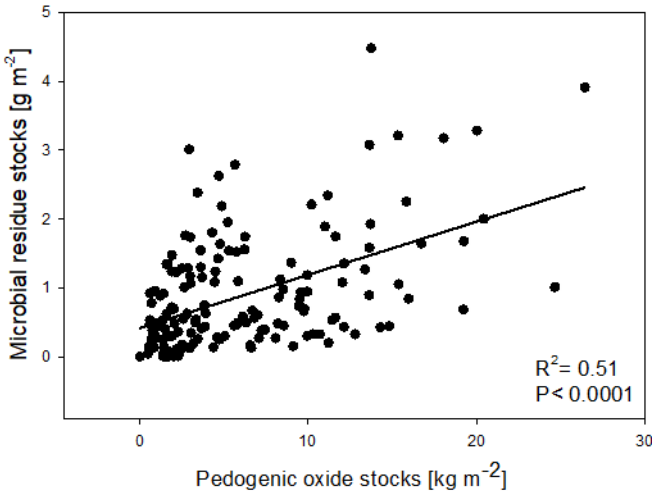

Fig.III 4: The correlation of microbial residue stocks with those of pedogenic oxides (sum of FeD AlD, MnD) indicates that organo-mineral interactions with oxides promote the accrual of microbial residue N

Paddy management effect in soils on Fe- and Al-rich substrates

We hypothesized that paddy management may affect N_t and microbial N sequestration to a higher degree in soils on Fe-rich substrates, due to frequent changes in redox conditions and thus a dissolution and precipitation of Fe oxides, which may then occlude OM. However, a positive effect of paddy management on N sequestration via microbial residues was significant only in Andosols and Chinese Alisols (the latter with relatively small Fe oxide contents). Especially for Javanese Alisols (rich in Fe oxides) we were surprised that paddy management effects on microbial N immobilization and subsequent storage in microbial residues was lacking, suggesting that other variables rather the mere flooding played a role for enhanced N sequestration. Such other variables may be differences in climate as well as specific management practices, such as the frequent intercropping with Chinese cabbage in paddy Andosols that elevates organic matter inputs to soil (Hill 1990). Such other variables may also comprise the use of clover as intercrop on paddy fields in China. It was found that after clover and ryegrass cultivation, the proportion of atmospherically derived N amounted to 60–80% of the total N content in soil (Høgh-Jensen und Schjoerring 1997). Another study showed that clover cultivation bound up to 43 kg/N/ha per year (McNEILL und WOOD

1990). Small $\delta^{15}N$ values in these Chinese Alisols support the assumption that large portions of N stemmed from N fixing plants rather than from decaying organic matter debris under paddy management.

Effects on clay-rich substrates

In soils developed on clay-rich substrates, larger clay contents promote SOM accrual (Six et al. 2000). The Vertisols contained 60% clay minerals, which likely contributed to the sorption of SOM and thus also of SON. Also self-ploughing and self-mulching effects contribute to SOM storage in Vertisols (Patra und Rego 1997). Although, Mikutta et al. (2007) noted that smectite dominated Vertisols are capable to stabilize organic matter, the Vertisols under study stored the smallest amounts of SON and microbial residues in our study (Fig.III 3). We conclude that the smectites were much less effective than Fe oxides in storing organic N forms.

In principle, the fine pores in clay-rich substrate may additionally retard the decomposition of OM due to an elongated period of oxygen deficiency in this fine grained material. However, we could not find a significant difference on Nt and amino sugar accrual between the topsoil of paddy managed and upland cropped Vertisols (Fig.III 3a), in the paddy managed subsoil the stocks of microbial residue N was even significantly depleted (Fig.III 3b). Hence, we suggest that we rather observed an effect of sugar cane cropping in the upland soils, which promotes N accumulation via biological N fixation (Lima et al. 1987, Boddey et al. 1995) as indicated by small $\delta^{15}N$ values (Fig.III 1a).

The sites in Italy were devoid of elevated clay contents. Yet, the total N and microbial residue accrual in the sandy Fluvisols were in similar magnitude as in other oxide-poor soils (Chinese Alisols and Vertisols), supporting our observations that pedogenic oxide content is an important factor in N sequestration via microbial residues, although climatic differences likely also add to different amino sugar accumulation (Amelung et al. 2006). Nevertheless, also at the Italian site, the soil under paddy management revealed larger N and microbial residue N stocks (Fig.III 2, Fig.III 3), suggesting that flooding leads to an accumulation of N in paddy soils also on fairly sandy substrate.

5 Conclusion

Here we asked whether paddy management and different substrates of soil influence the sequestration of N and especially N bound in microbial residues. We analyzed a series of soils from different substrates and found that the contents of pedogenic Al and Fe-oxides were the major control of N sequestration in microbial residues. This impact of oxides in soil overlayed even the effect of management, i.e. of frequent flooding with water, on total N and microbial N accrual.

IV.

BLACK CARBON AND BLACK NITROGEN UNDER LONG-TERM PADDY AND NON-PADDY MANAGEMENT IN MAJOR SOIL REFERANCE SOIL GROUPS

Modified on the basis of:

Black carbon and black nitrogen storage under long-term paddy and non-paddy management in major reference soil groups

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1 Introduction

Rice cultivation feeds about half of the human population (Maclean et al., 2002). Burning rice straw on the fields might significantly affect the carbon cycle. Gaddé et al. (2009) calculated for Asian countries (where most rice cultivation takes place) that 23 to 95% of rice straw residues are burned. This may contribute to atmospheric CO_2 and global warming, but residues of biomass combustion (black carbon, BC) may also contribute to relatively persistent C in soil (e.g., Seiler and Crutzen, 1980).

We previously showed that long-term paddy management on marsh sediments in China resulted in equilibrium BC accumulation and degradation. These Cambisols stored ~ 13 t BC ha^{-1} after 300 years of paddy management. Adjacent non-paddy systems had only 7 t BC ha^{-1} (Lehndorff et al., 2014). However, little is known about BC storage in other paddy soils, particularly if BC storage might systematically change in well-developed soils of different origins and mineral compositions.

Black carbon is recalcitrant in nature (e.g., Seiler and Crutzen, 1980). It comprises a range of incompletely burned organic matter (OM) characterized by fuel and combustion conditions (temperature and oxygen supply) (Czimczik et al., 2002; Keiluweit et al., 2010). Mean residence time (MRT) estimates in soil ranges from decades to millennia (e.g., Fang et al., 2014; Kuzyakov et al., 2014; Singh et al., 2012a). Differences in BC stability have been mainly related to the degree of aromatic condensation, which increases as burning temperature increases and depends on the fuel used (e.g., Singh et al., 2012b). On-field burning of rice straw or other crop residues yields BC equivalent to that of grass burned at about 300–400 °C (Lehndorff et al., 2014; Wolf et al., 2013).

Once incorporated into soil, the turnover of soil BC might additionally be controlled by interactions with minerals (Cheng et al., 2006; Cusack et al., 2012). For example, BC stocks in a weathering sequence of volcanic soils were closely related to the contents of reactive, short-range order minerals (Cusack et al., 2012). Incubation experiments also hinted that minerals may have a dominant role in BC turnover: BC in Ferralsols with high (crystalline) Fe oxide concentration had longer MRT (235–106 years) than BC in clay mineral-rich Vertisols (218–44 years for incubation temperatures of 20° and 40°C, respectively) (Fang et al., 2014). The authors concluded that variably charged oxide minerals stabilized BC better than the permanently charged smectites in the Vertisol. However, the authors also admitted that MRTs might differ dramatically under field conditions, due to other factors such as variations in climate conditions.

BC might also stabilize by occlusion by soil minerals (e.g., Brodowski et al., 2006). In paddy soils, changing redox conditions may lead to BC stabilization by reaction with, or occlusion in, frequently precipitated short-range order Fe oxides. Effects may vanish by reductive dissolution of Fe oxide-OM associations (e.g., Kirk, 2004; Winkler et al., 2016). In clay-rich soils, BC may be physically protected in deeper soil horizons upon swelling and shrinking (self-plowing due to deep cracks that develop in dry seasons, which allow fresh OM to directly enter the subsoil). These effects may alter the residence time of BC to an unknown extent. In contrast, Fang et al.'s (2014) incubation study implied that Vertisols contain less BC than Ferralsols, and at a more advanced stage of degradation. In any case, it seems reasonable to assume that the storage of BC in soil relates to its mineral assembly.

Amounts and composition of BC in soil can be estimated by a variety of methods, but each has limitations. These limitations are mainly due to large differences in the BC materials. Previous studies showed that oxidation of BC to benzene polycarboxylic acids (BPCAs) recovered at least 70% of BC as BPCA in soil (Hammes et al., 2007; Roth et al., 2012). The relative amounts of five- to six-times carboxylated BPCAs relate to the degree of aromatic condensation of BC in soil (McBeath et al., 2011; Schneider et al., 2010). This is a result of the variation in BC surface/interior ratios. In other words, the less the proportion of six-times carboxylated BPCA (mellitic acid; B6CA), the larger the surface of BC (Glaser et al., 1998). The composition of BPCAs may be related to the source of BC, indicating if it stems from biomass, such as rice straw burning, or industrial combustion processes (Lehndorff et al., 2015; Wolf et al., 2013). Additionally, it was assumed that BC degradation leads to large, oxidized, negatively charged surfaces that react with the soil matrix, thereby conserving BC (Brodowski et al., 2005a; Cheng et al., 2006). This effect may differ for aerobic (non-paddy) and anaerobic (paddy) management conditions. BC accumulation, especially in subsoil, may be accompanied by a relative loss of mellitic acid due to decondensation (Rodionov et al., 2010). Singh et al. (2012) reported that BC characterized by smaller proportions of mellitic acid was more prone to stabilization processes than condensed counterparts. In this line, both preferential stabilization of decondensed BC and intrinsic stability may contribute to its long residence time in soil (e.g., Singh et al., 2012a).

Burning OM also produces BN, but its role in N storage and cycling is not yet understood (de la Rosa and Knicker, 2011; Knicker, 2007). In previous studies, we found that at least 50% of paddy soil N was abundant in unidentified forms, or not bound to microbial residues (Roth et al., 2011) and proteinaceous amino acid N (unpublished data). X-ray photoelectron spectroscopy (XPS) may reveal if straw burning in paddy management leads to accumulation

of N in heterocyclic, aromatic forms as previously detected in other soils (Abe et al., 2005; Mikutta et al., 2009). For example, Ding et al. (2014) related the aromatic N peak in the XPS N 1s spectra to BN in water samples and found a coupling between dissolved BN and BC. We tested if the method is applicable to rice char and soil, and if there is a correlation of BC to aromatic N.

The main objectives of this study were to elucidate the possible effects of paddy versus non-paddy management, and different major reference soil groups on the storage of BC. We sampled pairs of paddy and adjacent non-paddy soil depth profiles from different major soil groups of the world (IUSS Working Group, 2014), analyzed them for BC, and compared this to pedogenic oxide contents and soil texture (see also Winkler et al., 2016). This inevitably included a sampling from different climatic and land-use regions, since soil mineralogical settings were specific to geographic regions and parent material. Since the accumulation of aromatic N in soil due to inputs of BN is not yet well understood, we aimed to detect parallels in soil BC and BN contents. Sampling was done for well-developed soils that were under permanent paddy and non-paddy management during at least the last century.

2 Materials and Methods

Sampling

For detailed information of sampling see chapter II 2. All information about individual site management, such as crop rotation, yield, fertilizer application and herbicides were gained by interviewing the local famers (for a summary see Tab.VII 1 in Appendix B).

Soil details are described next.

Java, Indonesia

The substrate at Java is mainly of volcanic andesitic origin, consisting of silicate minerals rich in Al and Fe, which form reactive, pedogenic oxides during pedogenesis. Rates of weathering and pedogenesis are generally high due to the tropical monsoon climate. The climate is more humid in West Java (Sukabumi and Jasinga) than in East Java (Ngawi), which has pronounced interchanges of dry and humid weather (Tab.VIII 1 in Appendix C). Soils at Sukabumi were Andosols under vegetable-maize (non-paddy) cropping and Al-rich Anthrosols under paddy-paddy-pak choi rotation (Tab.VIII 1). At Jasinga, Fe oxide-rich Alisols formed on andesite, which are now under agroforestry (non-paddy). The respective Hydragric Anthrosol was under rice-rice-maize rotation (Tab.VIII 1). Local farmers said that

on-site burning of straw residues and waste frequently occurred, except for the non-paddy site at Sukabumi (Tab.VIII 1).

The soils at Ngawi formed on fluvial clay deposits in a broad, shallow valley and were characterized as Vertisols (under sugar-cane monocropping) and as Hydragric Anthrosols under annual paddy-paddy-tobacco rotation. Swelling and shrinking caused formation of thick, deep cracks during the dry period. This allowed for translocation of topsoil material, including charcoal, to deeper soil layers. The non-paddy cropping system had intensive burning, while rice straw was partly harvested for livestock.

China

At the Red Soil Research Station (Yingtan), soil formation into limnic and fluvial sediments under subtropical climate conditions resulted in Fe-rich Alisols under non-paddy and Anthrosols under paddy-paddy-fallow management. Paddy management involved cultivating clover to increase soil N stocks approximately every five years. Both the paddy and non-paddy cropping systems had frequent on-site burning of straw residues for about 300 years.

Cambisols were sampled in the Yangtze River Delta in the Bay of Hangzhou. The soils developed on deltaic sediments after land embankment under paddy and non-paddy management, respectively. Paddy and non-paddy soils had undergone 700 years of crop management and frequent burning of straw residues. Buried topsoil horizons found in two subsoils were excluded from BC stock calculation (see Lehndorff et al., 2014).

Italy

At Zeme, Italy (Rice Research Centre of Ente Nazionale Risi), paddy management was established about 30 years ago in the flood plain of the Po River. Soils forming under temperate climates with seasonally high groundwater levels were Endogleyic Fluvisols under maize monocropping and Haplic Gleysols under paddy management with one rice harvest per year. Crop management did not include puddling and straw burning. Both soils were fallow during winter.

Soil properties (pedogenic oxide contents, texture, and structure)

The soils under study had varying physical and chemical properties. The Andosols had 24–61 g kg^{-1} oxalate-extractable Al (Al_o, likely from allophane), 8–16 g kg^{-1} oxalate-extractable Fe (Fe_o, short-range order Fe oxides) in their topsoil. All other topsoils had less than 4 g kg^{-1} Al_o. The Indonesian Alisols were characterized by 3.2–10.5 g kg^{-1} Fe_o, while the Chinese Alisols had 1.0–1.9 g kg^{-1} Fe_o. The Vertisols had 3.3–4.9 g kg^{-1} Fe_o in the topsoil horizons. Chinese

Cambisols had 1.3–4.6 g kg^{-1} Fe_o (Kölbl et al., 2014). The Gleysol/Fluvisol soil pair had 1.4–3.5 g kg^{-1} Fe_o (details of Fe and Al analysis in Winkler et al., 2016).

The abundance of clay was estimated by grain-size analysis (Winkler et al., 2016). The Indonesian Andosols were of silt loam, and Alisols had 15/45/40% sand/silt/clay (clayey Alisols). The Chinese Alisols (sandy Alisols) and the Gleysol/Fluvisol soil pair from Italy had > 50% sand-sized particles. Vertisols had 50 to 70% percent clay-sized particles in the topsoil. The Chinese Cambisol had about 70% silt and 25% clay. Soil structure was subangular-blocky for all soils except for the Vertisols, which had wedge-shaped aggregates. The Gleysol/Fluvisol pair had a massive structure in the subsoil.

Analyses

Bulk density (BD) and sample pre-treatment

Undisturbed soil cores (100 cm^3, in triplicate) were taken from each horizon under field-moist conditions to determine the BD (Black and Blake, 1965). In addition, composite samples were taken for each horizon from different parts of the profile pit and air-dried. All soil samples were sieved to < 2.0 mm and ground in a ball mill prior to BC analysis.

Carbon, organic carbon, and nitrogen

Total carbon and nitrogen of each sample were determined by dry combustion at 950 °C using a Vario Max elemental analyzer (Elementar Analysensysteme, Hanau, Germany). The inorganic carbon content was determined by dissolution of carbonates with phosphoric acid and subsequently detecting the evolving CO_2 (C-MAT 550, Ströhlein GmbH, Viersen, Germany). For this purpose, ground samples were placed into gas-tight reaction vessels, 42% phosphoric acid was added, and the suspension was stirred. A steady flow of N_2 transports the evolving CO_2 into an infrared detector. All analyses were carried out in duplicate. Concentrations of soil organic carbon (SOC) were calculated by subtracting inorganic carbon from total carbon.

Oxidation of black carbon to benzene polycarboxylic acids (BPCA)

The amount of BC in soil can be estimated by oxidation to BPCAs (Glaser et al., 1998). The BPCA oxidation was performed according to a modified method (Brodowski et al., 2005b). Sample aliquots, equivalent to a maximum of 5 mg OC (Kappenberg et al., 2016), were hydrolyzed with trifluoro-acetic acid for metal elimination (105 °C, 4 h). The residue was oxidized with 65% HNO_3 (170 °C, 8 h). BPCAs were then purified on a cation exchange column (Dowex 50 W X 8, 200–400 mesh, Fluka, Steinheim, Germany). The individual

BPCAs were then converted to trimethylsilyl derivatives, separated by gas chromatography on an Optima-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Macherey-Nagel, Düren, Germany), and detected via flame ionization (Agilent 6890 gas-chromatograph). Citric acid was used as the first internal standard for BPCA quantification and added immediately before the cation exchange step. Biphenylene-dicarboxylic acid was used as a second internal standard to quantify the recovery of citric acid, which was 70–100%. The sum of two- to six-times carboxylated BPCAs was corrected for CO_2 loss and insufficient conversion of BC to BPCAs by a factor of 2.27 (Glaser et al., 1998), representing a conservative minimum estimate of BC (Brodowski et al., 2005).

Information on the degree of aromatic condensation of BC was achieved by relating the percentage of 4- and 5-times carboxylated acids (B4CAs = sum of prehnitic, mellophanic, and pyromellitic acid, B5CAs = benzenepentacarboxylic acid) to six-times carboxylated acids (B6CAs = mellitic acid). BC stocks [t ha^{-1}] were calculated from BD and horizon thickness for topsoil and down to 1 m depth for total BC stocks.

Black nitrogen (BN) analysis by X-ray photoelectron spectroscopy (XPS)

Straw, burned straw, and topsoil horizons (0 to a max. 15 cm) were analyzed for their bulk chemical surface composition using a Kratos Axis Ultra DLD instrument (Kratos Analytical Ltd., Manchester, UK). As most C and N in soil are enriched on particle surfaces, this analysis was done to detect even small contributions of BN. To also identify BN located within small aggregates otherwise not accessible by XPS, we analyzed homogenized samples over a large spatial area, which gave us information about average N species in a given soil sample. Homogenized powders of source materials, deposited onto adhesive copper-nickel tape, were analyzed for survey and N 1s detail spectra at three positions using monochromated AlKα radiation with excitation energy of 1486.6 eV. Spectra were recorded in hybrid lens mode, with photoelectrons collected from an area of each 300 × 700 µm. Acquisition parameters for survey spectra were: Pass energy, 160 eV; step size, 1 eV; three sweeps. Parameters for N 1s spectra: Pass energy, 20 eV; step size, 0.1 eV; three sweeps. After charge correction (survey C peak set to 285 eV), the N 1s peak was deconvoluted into three subpeaks representing different N oxidation states by using the Unifit 2010 software package (Hesse et al., 2003). The background was fitted together with the three subpeaks to obtain best-fit results. The following N types were distinguished (Mikutta et al., 2010): N bonded in aromatic structures (398.8 ± 0.4 eV), including imine, heterocyclic C=N, and aromatic amines; N in peptide bonds (400.5 ± 0.2 eV), including pyrrole and secondary and tertiary amines, and imides; and primary amine N, including protonated amines (402.4 ± 0.1

eV) (see Fig. S1). Fitting was accomplished by a Lorentzian-Gaussian mixing ratio of 0.2 and constraining the full-width-at-half-maximum values between 0.5 and 2.5. Atomic% element concentrations were converted into mass-based concentrations by taking into account the molar weight of each element (Gerin et al., 2003).

Statistics

Differences in SOC contents, BC amount, and composition (topsoil and total BC stocks [t ha^{-1}] and B5CA/B6CA ratio) were tested between soils (ANOVA) and for the combined effect of paddy and non-paddy management in the different soils from major reference soil groups (Andosols, Alisols [Indonesia], Alisols [China], Vertisols, Cambisols, Fluvisol/Gleysol n = 6; MANOVA). Normal distribution, tested by a Shapiro-Wilk-Test, was just reached (n = 36 and W = 0.942 and 0.940, respectively). Post-hoc Tukey HSD tests highlighted individual differences between management forms and/or soil groups. These analyses were done with STATISTICA 8.0 (StatSoft, Inc.). Linear regression was tested for BC [g C kg C_{org}^{-1}] versus aromatic N [%], clay-sized fraction [%], and Fe_o [g kg^{-1}] using SigmaPlot 11 (Systat Software, Inc.)

3 Results

Soil organic carbon (SOC)

The largest SOC concentrations were found in Andosol topsoils, and the smallest concentrations were found in the Fluvisol/Gleysol pair (Fig.IV 1, Tab.VIII 2 in Appendix C). These two soil groups contributed the greatest share of the difference between all topsoils, which was significant at the $p<0.0001$ level. Mean SOC concentration of two topsoil horizons (in non-paddy soils), three topsoil horizons (in paddy soils, including plow pan), and three field replicates were 39.7 ± 4.3 g kg^{-1} in paddy (P) and 35.9 ± 1.9 g kg^{-1} in non-paddy (NP) Andosols, followed by Alisols (Indonesia P: 17.1 ± 3.7, NP: 22.5 ± 2.0 g kg^{-1}; China P: 17.8 ± 11.8, NP: 7.1 ± 0.1 g kg^{-1}), Cambisols (P: 16.6 ± 1.4 and NP: 9.1 ± 1.9 g kg^{-1}), Vertisols (P: 11.7 ± 3.3, NP: 14.4 ± 1.0 g kg^{-1}), and the Gleysol/Fluvisol pair (P: 9.9 ± 3.2, NP: 7.2 ± 0.5 g kg^{-1}). Management affected SOC contents at a $p <0.0001$ level, and a post-hoc analysis highlighted the difference between Alisols and Cambisols from China ($p<0.0002$). The SOC concentrations decreased with soil depth in all profiles. Subsoil OC contents ranged between 24.6 g kg^{-1} in Andosol subsoils under paddy management and 0.5 g kg^{-1} in Fluvisol subsoil under non-paddy management (Tab.VIII 2 in Appendix C). The depth gradient of SOC was

most pronounced in Andosols, Alisols and Cambisols, but less developed in the Vertisols and the Gleysol/Fluvisol pair (Fig.IV 1). A management effect (paddy versus non-paddy) on the depth distribution of SOC was not observed.

Black carbon (BC)

Average BC concentrations in topsoil horizons ranged from 2.6 ± 0.3 to 0.4 g BC kg^{-1} soil, with the largest values in Andosols under paddy management and in Vertisols (Tab.VIII 2 in Appendix C). Paddy management affected topsoil BC concentrations in Andosols, Alisols (China) and Cambisols toward larger BC contents in paddy soils ($P < 0.002$) and toward smaller BC contents in Vertisols under paddy management ($P < 0.006$; Table 2; Table S1). Differences related to soil group were significant ($P < 0.0001$), which was especially driven by the Vertisols and the Fluvisol/Gleysol pair, since these soils had both contrasting BC contents and little variation between management forms (Tab.VIII 2 in Appendix C). The BC concentrations decreased with depth in all profiles and varied between 0.1 and 1.7 g BC kg^{-1} in > 90 cm depth.

The variation in BC relative to SOC for topsoils was 189 ± 57 to 27.9 ± 2.4 g BC kg^{-1} SOC, with the largest BC portions in the topsoil layers of the Vertisols (Fig.IV 1). In the Andosols and Alisols, BC tended to decrease with depth relative to SOC, while in Vertisols, paddy-managed Cambisols and the Gleysol/Fluvisol pair, BC accumulated relative to SOC at larger soil depth. Maximum relative BC enrichment was found in the subsoils of the paddy Cambisols and Vertisols, amounting to more than 30% of SOC, which was almost double the portion found in the respective topsoils (Fig.IV 1; buried topsoil horizons in Cambisols excluded). Andosols, Vertisols, and Alisols (China) indicated differences in BC enrichment relative to SOC upon paddy management ($p<0.04$). However, paddy management for the Vertisols led to significantly lower BC proportions ($p<0.0005$; Fig.IV 1). Differences between soils were sustained at $p<0.0001$ for BC/SOC. However, Alisols (China and Indonesia) did not differ significantly from Cambisols.

Total BC storage differed for the soils under study (stocks for 1 m depth, $p < 0.0001$). Vertisols had the largest total BC stocks of 17 and 19 t C ha^{-1}, followed by Cambisols with 13 t ha^{-1} (Fig.IV 2). These two soils contributed the most to the difference between soils (post-hoc test). Andosols under long-term paddy management accumulated a total of 8 t BC; Alisols had 6–10 t BC ha^{-1}. Non-paddy soils and paddy soils without rice straw burning (non-paddy Andosols and the Gleysol/Fluvisol pair) only had 3–4 t BC ha^{-1} (Fig.IV 2). A significant effect of paddy management on total BC stocks, i.e. affecting the soil profile down

to 1 m, was only found in Cambisols from China ($p<0.0001$). For topsoils, management significantly affected BC stocks in Andosols and Cambisols ($p<0.001$). The Vertisols showed no significant effect of management, but total BC stocks differed from all other soils ($p <0.03–0.0004$).

Composition of individual BPCAs was dominated by benzenepentacarboxylic acid (B5CA) and four-times carboxylated acids (B4CAs). The relative share decreased in order: B5CA (24–100, average 36%) > B4CA (0–70, average 33%) > B6CA (0–42, average 31%) > B3CA (0–7, average 4%). B5CA dominated in all topsoils, except for the topsoils of Alisols and paddy Cambisols in China, which were dominated by B6CA (B5CA/B6CA ratio < 1, Fig. 2, $p < 0.0001$). Topsoil BPCA composition was not affected by management practice ($p>0.5$). Variable contributions of BPCAs were observed with soil depth, but were small (B5CA/B6CA ratio in Fig.IV 1) and not specific for soil groups or management practices ($p>0.5$). The Gleysol/Fluvisol pair had increasing proportions of B5CA in the subsoils, increasing to 100% (Tab.VIII 2 in Appendix C). However, the absolute amount of B5CA was only about one-fifth of that observed in the other soils (data not shown), so most of the other BPCAs were likely below the detection limit.

In summary, when compiling information from both SOC and BC data, we found effects of both management and soil-group. Management effects specially affected absolute concentrations of BC in topsoil (of Andosols, Vertisols, Chinese Alisols, and Cambisols). Detection of soil-specific differences was improved by looking at relative contributions of BC to SOC in topsoils and complete profile stocks.

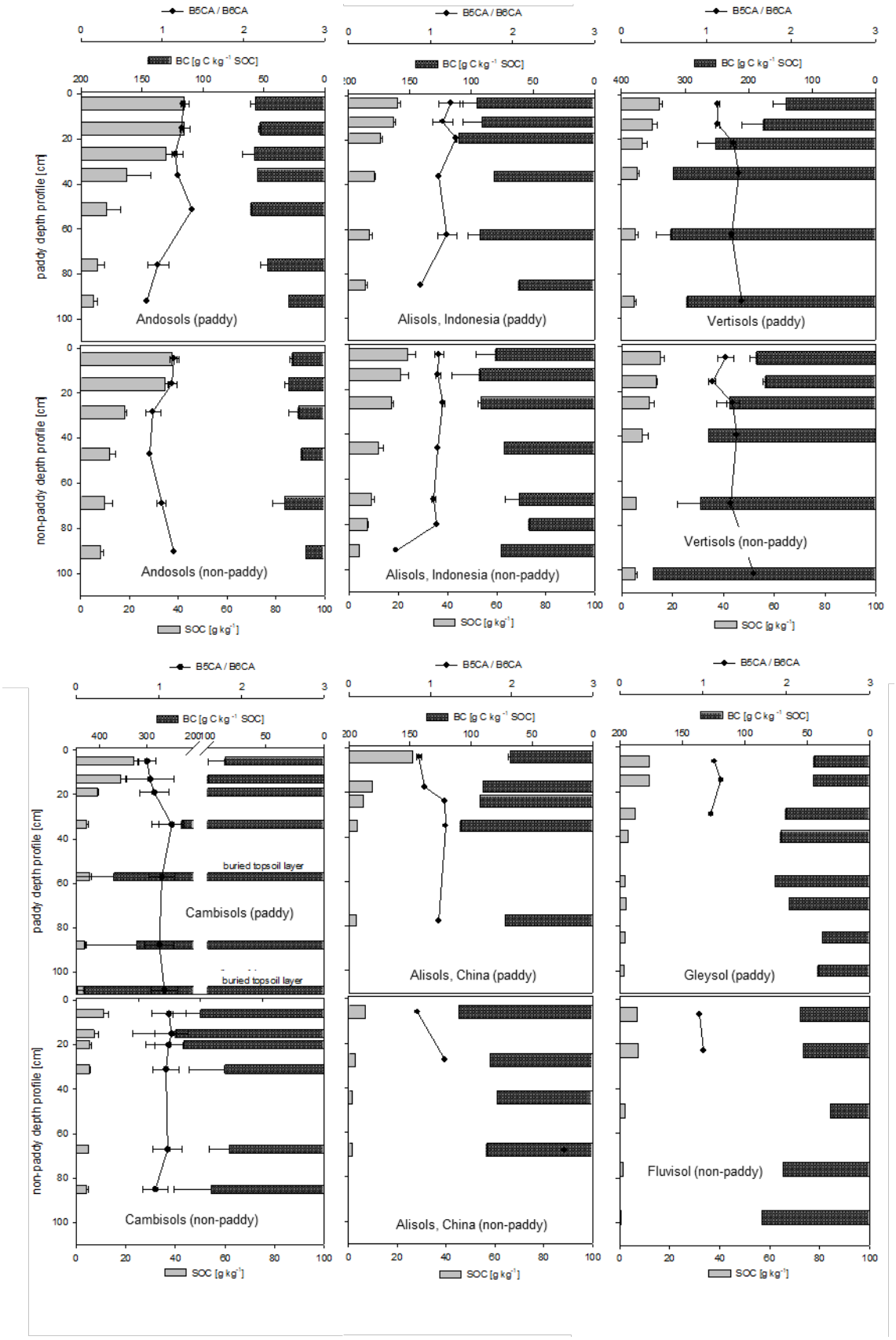

Fig.IV 1: Soil organic carbon concentrations (SOC) (left), black carbon (BC) (right), and the ratio of pentacarboxylic to mellitic acid (B5CA/B6CA) in depth profiles of different paddy and non-paddy soils (averaged across field replicates, for subsoils only one horizon was replicated, for Chinese Alisols only topsoil horizons were replicated; Gleysol/Fluvisol was sampled from one site only (error bars indicate SD; BC calculated from ∑ BPCA * 2.27).

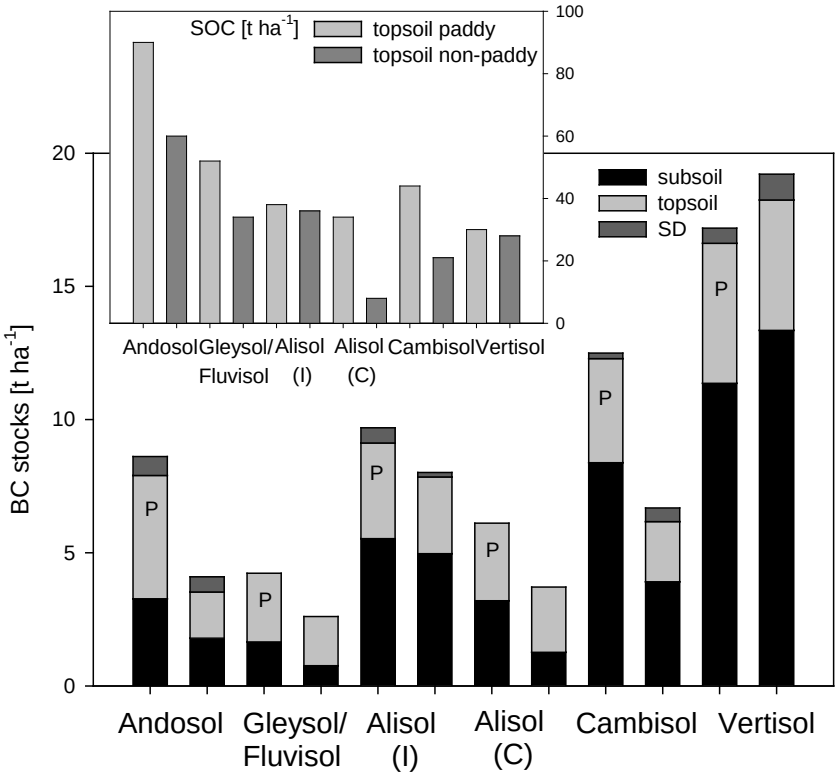

Fig.IV 2: Total and topsoil BC stocks of different soil groups under paddy (P) and non-paddy management. The stocks were estimated from oxidation of soil BC to benzene polycarboxylic acids from three field replicates per site (except for Chinese Alisols and the Gleysol/Fluvisol pair; C = China, I = Indonesia; BC calculated from ∑ BPCA * 2.27).

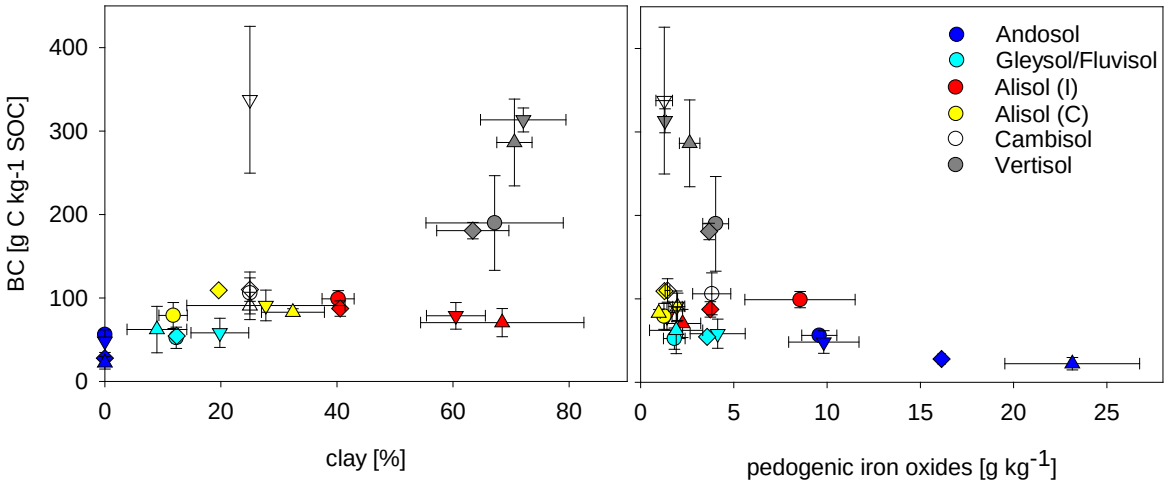

Fig.IV 3: Relative soil BC content vs. a) clay-size fraction, and b) oxalate-extractable Fe. Dots and diamonds represent the average for paddy and non-paddy topsoil horizons. Triangles facing down and up indicate paddy and non-paddy subsoil, respectively (average of three field replicates and different soil horizons, except for Chinese Alisols and the Gleysol/Fluvisol pair; clay-size fraction analyzed for one profile per site only, C = China, I = Indonesia; BC calculated from ∑ BPCA * 2.27).

Nitrogen and black nitrogen (aromatic N)

The XPS-derived N concentrations in rice straw represented N concentrations at particle surfaces and were higher than those determined by bulk element analysis (10 g kg^{-1} and 3.8 g kg^{-1};Tab.VIII 3 in Appendix C). In soil samples, XPS-N was 2.3–13.7 g kg^{-1}, while element analysis yielded 0.7–4.2 g N kg^{-1}. A moderate correlation was observed between N contents obtained by the two methods (R^2 = 0.52;Tab.VIII 3). Black N was detected as aromatic N at ~399 eV. Aromatic N represented 5% of total N in rice straw and was enriched to 57% in rice char (Fig.VIII 1 in Appendix C). In soils, aromatic N contributed only up to 8% to total XPS-derived N. Although variability in the XPS signal was high ("noise", Fig.VIII 1 in Appendix C), Abe et al. (2005) were able to show by complementary ^{15}N CPMAS NMR analysis that under similar analytical conditions at least 5% aromatic N from soil can be detected. The highest aromatic N proportions occurred in the Italian soils. Surprisingly, these soils had the lowest total N and BC contents.

4 Discussion

Biomass input and turnover controlled accumulation of SOC in topsoils. Paddy management led to almost double the amount of SOC stocks in topsoils of Cambisols compared to non-paddy cropped soils, which was likely related to reduced turnover of SOC under anaerobic conditions in paddy fields (Kalbitz et al., 2013). The Chinese Alisols in our study also showed significant increases in SOC concentration under paddy management ($p<0.0001$) (Winkler et al., 2016), while Indonesian soil samples showed no significant response of topsoil SOC to paddy management.

Paddy management typically produces a very dense plow pan, potentially leading to a reduced translocation of SOC into the subsoil (Kögel-Knabner et al., 2010), as observed in Chinese Cambisols in a previous study (Kalbitz et al., 2013). Here, depth profiles of SOC indicated that OM input into subsoils was reduced by paddy management for Andosols and Chinese sandy Alisols, but not for Indonesian clayey Alisols, Vertisols, and the Fluvisol/Gleysol pair (Fig.IV 1). This fits to other observations, such as that the paddy plow pan did not limit C inputs into the subsoil in young coarse-textured soils (Said-Pullicino et al., 2015). In contrast, a layer of cemented Fe oxides likely restricted the transport of SOC down to the 40 to 50 cm depth in paddy-managed Andosols (own observation).

Stabilization and accumulation of SOC in Andosols have been attributed to binding to aluminosilicates (allophones), gibbsite, and Fe oxides (Junet et al., 2013). Winkler et al.

(2016) showed that Fe oxide, phyllosilicates, clay minerals, and allophanes did not directly affect SOC in paddy managed soils. Leaching these potential OC accumulators from paddy-managed topsoils did not reduce SOC. The authors assumed that differences in crop residue input between paddy and non-paddy soils were more relevant. The following section will discuss if and to what extent BC accumulation was driven by the soil mineral assembly in paddy managed soils and by other soil specific properties, such as soil texture.

Black carbon sources

Burning of crop residues for paddy- and non-paddy managed sites was the major source of BC, except for the non-paddy Andosols and the Italian site, where no burning was reported. The amount of burned rice straw likely varies. We received information that crop yields differed from 6–12 t ha^{-1} and year, and were highest at the Vertisol sites. However, at this site rice straw is rather used for livestock breeding (as fodder and litter) than for on-field burning, which likely reduces the effect of higher yields on BC input. Although information about crop yields and burning practices are vague, enhanced BC input was reflected by elevated BC concentrations in soils at sites subject to intensive burning of crop residues. For example, the Andosols with rice-straw burning had absolute and relative BC contents exceeding those of the unburned, non-paddy Andosols by a factor of > 2 ($p<0.0001$). The unburned Gleysol/Fluvisol soil pair had only about one-fourth the absolute BC concentrations found in the other soils. Nevertheless, the unburned sites (non-paddy Andosol and the Gleysol/Fluvisol pair) also had BC in the soil, likely from atmospheric deposition.

The composition of BPCAs in topsoil may indicate the source of BC. It differed between Indonesian topsoils and the Chinese and Italian site (B5CA/B6CA ratio Fig.IV 1; $p<0.0001$). For the Indonesian sites the ratios ~ 1.1 were comparable to that found for burned straw in previous studies (Lehndorff et al., 2014; Roth et al., 2012; Schneider et al., 2010). The Chinese Alisols and Cambisols had B5CA/B6CA ratios <0.9 (Fig.IV 1). That ratio value indicates that about 10% of the BC was likely from burned fossil fuel, which produces high shares of mellitic acid (B6CA; calculation based on assumption that BC stemmed from diesel combustion with a B5CA/B6CA ratio ~0.15) (Roth et al., 2012). However, most of the BC at all sites was from crop-residue burning.

Management and soil specific effects on BC storage

Black carbon in Andosols

Indonesian Andosols were predicted to have the greatest potential for BC storage, since they had high concentrations of Al and Fe oxides and thixotropic properties. The latter indicated the presence of reactive nano-sized minerals such as allophanes and ferrihydrite. Allophane-type minerals and Fe oxides are known for their large sorption of OM (Batjes, 1996; Yamaguchi and Okazaki, 2002). Absolute amounts of SOC and BC were largest in the Andosols, indicating strong accumulation and probably stabilization of SOC, including BC. In Andosols from intermediate to felsic parent material, 70% of the SOC was found in organo-mineral complexes, half of that bound to alumosilicates, and only small proportions associated with Fe oxides (Junet et al., 2013). In Andosols formed from basaltic parent material, accumulation and stabilization of OM was mainly due to short-range order Fe oxides or ferrihydrite; aromatic compounds, likely from vegetation fires, occurred in mineral-organic associations (Mikutta et al., 2009). Cusack et al. (2012) also found a large, concomitant accumulation of BC and SOC in Andosols, suggesting that BC was co-stabilized with SOC by reactive mineral phases.

Andosol subsoils were characterized by Fe oxide-cemented layers at the 40-50 cm depth, restricting OM translocation. This was indicated by strongly decreasing SOC concentrations (Fig.IV 1). The BC concentrations decreased (Tab.VIII 2 in Appendix C) even more strongly than did SOC (Fig.IV 1), pointing at differences in BC and SOC translocation. SOC might have been transported in soil water through small pores in the Fe pan and along the surfaces of Lahar fragments (volcanic debris flow), while BC was barely transportable with water in other soil profiles (e.g., Major et al., 2010). While OM maybe transported with water in dissolved form in the soil column and associated to nanoparticles (e.g. Kaiser et al., 1996; Gottselig et al., 2014), little is known about leaching of BC through soil in nanoparticulate forms.

Research has frequently discussed BC degradation in soil. A control by biotic (e.g., Kuzyakov et al., 2014; Singh et al., 2012a; Zimmermann et al., 2012) and abiotic processes (e.g., Ward et al., 2014) was discussed in incubation studies. Field observations showed a trend to less-condensed BC forms (loss of B6CA) in depth profiles of grassland soils. This may be related to BC degradation (Rodionov et al., 2010), e.g., highly condensed BC particles degrade to less condensed, smaller aromatic particles. Here, B4CA, representing the surface portion of BC, relatively increased from the 50 cm depth downward at the expense of B5CA and B6CA (representing the interior portion; Tab.VIII 2 in Appendix C), supporting this degradation

concept. However, it is also possible that old BC deposited with the Lahar led to the observed differences in BC composition.

We assumed a paddy-management effect on SOC and BC stocks in the Andosols due to: A larger input of BC at the paddy fields, since non-paddy fields were not burned; anaerobic conditions that slowed down SOC and BC turnover (Kalbitz et al., 2013; Lehndorff et al., 2014); and changing redox conditions in the paddy fields, leading to enhanced interactions of SOC and BC, with hydrous Fe oxides freshly forming during oxic phases. Although BC accumulation was significantly higher in paddy-managed soils than in those under non-paddy management, this was related to the missing input in the non-paddy counterparts (see discussion above) rather than an effect of paddy-associated changes in redox conditions on organo-mineral complexes. This conclusion is supported by the lack of differences for SOC contents (Tab.VIII 2 in Appendix C) and a missing correlation between BC and Al, and Fe oxides (Fig.IV 3).

Black carbon in Alisols

We assumed that SOC and BC stabilization in Alisols would also be due to bonding to, or inclusion, in Al and Fe oxides (Fang et al., 2014; Kaiser et al., 1996). For example, low pH values in Alisols contributed to formation of strong organo-mineral associations through ligand-exchange reactions (e.g., Fang et al., 2014). In an incubation study employing four different soils, those soils richest in Fe oxides (Ferralsol) stabilized most of the applied biochar (Fang et al., 2014). Concentrations of SOC and BC in the topsoils of the studied Alisols were large and similar, except for the non-paddy-managed soil from China ($p>0.07$). However, a weak correlation of BC enrichment relative to SOC and Fe_o was observed for the Indonesian Alisols (R^2 0.16, $p<0.03$; Fig.IV 3). Still, the SOC in Alisols was more enriched in BC than that of Andosols. Therefore, other mechanisms than mere association to Fe oxides determine the amount of BC found in soil, such as differences in BC input or soil texture.

Depth profiles of SOC in the Indonesian and Chinese Alisols were less steep than in the Andosols. This can be related to a more homogeneous and non-cemented matrix, allowing for better vertical transport of substances, together with the probably small retention capacity of topsoils (due to high shares of crystalline oxides), which promotes deeper movement of OM. The more sandy texture of the Chinese Alisols likely contributed to the low SOC contents in the subsoil, which in turn elevated the overall contribution of BC to SOC. Nevertheless, the overall contribution of BC to SOC also decreased with depth. This indicated a lack of BC input to the subsoil, as discussed for the Andosol above, and does not support the general idea of selective preservation of BC during SOC mineralization. In the non-paddy subsoil, BC

contents were even close to the detection limit. Mellitic acid (B6CA) in particular was sometimes not detectable (Tab.VIII 2 in Appendix C, Fig.IV 1). One peculiarity about BPCA composition of the Chinese Alisols and the Cambisols discussed above was the B5CA/B6CA ratio values <1, indicating the presence of some BC from fossil fuel combustion. We may take advantage of that contribution and conclude from the abrupt change at the plow layer that BC was not significantly translocated deeper than 20 cm into the subsoil. This agrees with a biochar study in an Oxisol, which showed that BC leaching occurred only at the 30 cm depth (Major et al., 2010).

In summary, paddy management only affected BC contents of Alisols in China. Region-specific differences in management, such as straw burning, seem to be the controlling factor of BC in Alisols, as this was also practiced on the non-paddy Alisols in Indonesia. An effect of paddy-specific changes in redox conditions on BC storage in Alisols could not be found.

Black carbon in Vertisols

Vertisols showed the typical morphological features of soils rich in expandable clays, such as large cracks and bumpy surfaces (beside the agricultural plots) and large wedge-shaped aggregates formed due to soil swelling and shrinking with changing moisture. This strong peloturbation can cause OM incorporation into deeper soil horizons (Hulugalle and Entwistle, 1997). However, SOC concentrations in Vertisols were low, possibly due to the lack of biomass return, the low sorption capacity of smectites toward organic anions, and possibly more effective mineralization of OM in the dry period along desiccation cracks (Bundt et al., 2001). The lack of SOC accumulation may also explain extremely high proportions of BC in these soils, as it was selectively preserved (Brodowski et al., 2007). Residual biomass was frequently burned in sugar cane fields, and its BC was incorporated almost homogeneously into at least the 115 cm soil depth (Tab.VIII 2 in Appendix C; Fig.IV 1). Although we were told that rice straw was traditionally used as fodder in this region of Indonesia, BC contents were also elevated in the paddy soils (but to a smaller degree; $p<0.005$). This management effect became insignificant when looking at total and topsoil BC stocks. Peloturbation seemed to lead to a notably larger BC accumulation in Vertisol subsoils than in other soils (Fig.IV 1).

The incubation study by Fang et al. (2014) showed that the MRT of BC in a Vertisol was the shortest of the four tested soils (young soils to weathered Ferralsols and Vertisols). Again, BC storage in the field contradicted laboratory findings. Possible reasons might be the BC input into the soil via peloturbation, which cannot be accounted for in laboratory studies, stabilization by physical enclosure in aggregates or sealed cracks, and site-specific differences in climate or burning practices during the last decades. In any case, BC degradation rates and

related mechanisms derived from laboratory experiments do not allow for proper estimation of BC contents in the field. Indicators of BC degradation were lacking. In other words, the BPCA composition remained stable throughout the paddy and non-paddy Vertisol profiles, with dominance of B5CA (35–40%). Also there was no effect of paddy management on BC contents. However, the non-paddy fields were monocropped with sugar cane. The type of land use involves intensive burning of crop residues as well.

Black carbon in Gleysols/Fluvisols

The Gleysol/Fluvisol pair in Italy was sampled as a reference site with no straw burning. The BC contents suggested that atmospheric BC inputs formed a background of at least 0.5 g BC kg^{-1} soil, which is about 20% of what was accumulated in the burned soils and 50% of that in the unburned Andosols (Tab.VIII 2 in Appendix C). Although sampled in an industrial region, this background was smaller than that in other soils in remote areas in Europe (~1 g kg^{-1} BC; Nam et al., 2008). Overall, the small background BC contents indicated that straw burning elevates BC levels in soil much more than atmospheric inputs.

Gleysols are often enriched in SOC due to input of dissolved OM in the oxidized horizon mobilized under anoxic conditions in adjacent settings and the slowed decomposition of OM under water-saturated conditions. Indeed, SOC concentrations were higher in the paddy-managed Gleysols, likely due to both the groundwater input and the seasonally water-saturated topsoil. Riedel et al. (2014) and Said-Pullicino et al. (2015) showed that reductive dissolution of Fe and Mn oxides in a gleyic Fluvisol caused preferential release of aromatic carbon forms similar to BC. This process, together with vertical fluxes of DOC into the subsoil in these coarse-textured soils might favor BC enrichment at a 20–42 cm depth (Fig.IV 1) (Said-Pullicino et al., 2015). Overall, BC contents in the Gleysol/Fluvisol pair were low, and detection limits for BPCAs were even reached for subsoils (Tab.VIII 2 in Appendix C).

Black carbon in Cambisols

The BC accumulation in Cambisols under paddy and non-paddy management was previously discussed in detail (Lehndorff et al., 2014). Burning was practiced at all sites, leading to a BC contribution to SOC of about 10% in the topsoil horizons (Tab.VIII 2 in Appendix C). Compared to other soil groups, topsoil BC concentrations ranged second together with the Indonesian Alisol (Fig.IV 1). Clay and Fe oxides contents were comparable to Chinese Alisols (Fig.IV 3). The main differences were pH, which was almost neutral, and the silty texture (75% silt) (Kölbl et al., 2014). However, a prominent factor for BC stabilization could not be shown. The subsoils of these Cambisols had BC contents even larger than those of the Vertisols (Fig.IV 1), likely due to burial of former topsoil horizons (Lehndorff et al., 2014).

We considered BC enrichment in Cambisol subsoils as an exceptional case, partly independent from soil properties.

Mineralogical controls on BC storage

Overall, total BC storage was soil-specific ($p<0.0001$), but this depended on multiple factors. For Andosols and Alisols, we expected a control of redox-processes on BC storage. In other words, we expected to find elevated BC contents in paddy soils that greatly depend on frequent changes in aerobic/anaerobic conditions. Although elevated stocks in paddy-managed topsoil were found for Andosols (Fig.IV 2), other parameters, such as BC input, were responsible for this accrual. We showed that Vertisols and Cambisols exhibited significantly different and greater BC storage potential compared to other soils (Fig.IV 2). Large BC stocks in Vertisols could be related to effective translocation of BC into the subsoil by peloturbation. The BC stocks of the tested Cambisols were affected by buried topsoil horizons, so may not be representative for this soil group. Surprisingly, subsoil BC accumulation greatly varied and was mainly responsible for the differences in total BC stocks between the soil groups under study (Fig. 3). Rodionov et al. (2010) found a range of 15–35 t BC ha^{-1} in depth profiles of grassland chernozemic soils, which also had the largest shares of BC stored in the subsoil. Topsoil BC stocks differed less. Burned Vertisol, Andosol, and Alisol stocks were statistically not different and ranged from 3.6–5.3 t BC ha^{-1} (Fig.IV 2).

In line with this sequence of BC storage, the abundance of clay-sized minerals in all soils had the highest correlation with BC enrichment (BC in g C kg^{-1} SOC, $R^2 = 0.5$, $p<0.0001$;Fig.IV 3). However, this was mainly due to Vertisol horizons. In contrast, in subsoils of the Indonesian Alisol, where clay contents were also high, BC proportions were low. The correlation with BC and Fe oxides for all soils was reciprocal (Fig.IV 3). A correlation was only found for top- and subsoil horizons for Vertisols (less BC/more Fe oxides in the topsoil) and on paddy- or non-paddy-managed Andosols (less BC/more Fe oxides in the non-paddy cropping system; Fig.IV 3). A few other studies showed that Fe and BC dynamics are coupled, especially with varying water saturation (Riedel et al., 2014). Nevertheless, we found only a weak positive correlation of BC to Fe oxides for the non-paddy-managed Alisols (Indonesia) ($R^2 = 0.55$; Fig.IV 3). Again, this contrasts with Fang et al.'s (2014) laboratory findings that high Fe contents would promote BC enrichment. Possibly Fe oxides may rather promote overall SOC enrichment, thus, diluting the overall contribution of BC to SOC. It became obvious in our study that BC input may strongly control BC storage. How this

masked the effects of Fe oxides on BC storage in paddy soils should be clarified in future studies.

Black nitrogen formation and accumulation in soil

Accumulation of aromatic N forms in soil has been related to inputs of burned OM (Knicker, 2007). We found that combustion of rice straw increased the proportion of aromatic N by a factor of approximately 10 to more than 50% relative to amide and primary N, while total N yields were halved (Tab.VIII 3;Fig.VIII 1 in Appendix C). It seems reasonable to assume that input of such rice char would increase aromatic N in soil, as it also increased BC. However, we found no systematic correlation between BC and aromatic N. Instead, soils with low BC/SOC contents and low N contents (Andosols, Gleysol/Fluvisol) were relatively enriched in aromatic N (see Andosol in Fig.VIII 1 in Appendix C). Although a high signal to noise ratio in the XPS spectra may have affected aromatic N quantification (Fig.VIII 1 in Appendix C), a lacking clear shoulder in the spectra at 399 eV suggests low aromatic N contents. We hypothesize that aromatic N is rather a product of ageing of OM in soil (Schulten and Schnitzler, 1997). This is further supported by observations made for the Vertisols, which showed the highest BC/SOC contents but no detectable N forms (Fig.VIII 1 in Appendix C). We discussed that this soil accumulated fresh OM via the peloturbation typical of that soil. This fresh OM likely completely diluted total and aged aromatic N contents. It remains open what happened to the aromatic N from rice char. Some studies suggest that BN degrades significantly quicker than does BC (Hilscher and Knicker, 2011). The soil mineral assembly was also unlikely to control the storage of aromatic N, since soils with the most (Andosols) and least reactive phases (Fluvisol/Gleysol) seemed to have the highest aromatic N portions (Tab.VIII 3 in Appendix C). Consequently, we suggest that aromatic N forms in soil and BN may have different sources, properties, and turnover than BC.

5 Conclusion

We found that crop-residue burning significantly increased BC stocks in arable top- and subsoils, leading to a maximum accumulation of 19 t BC ha^{-1} in Vertisols. Unburned sites had the smallest BC stock levels (< 4 t/ha). The BC stocks varied within soil groups, from Vertisols > Andosols > Alisols > Gleysols/Fluvisols. The BC accumulation in the subsoil was more specific for individual soil groups than that in the topsoil. Vertisols had the highest BC stocks, mainly due to effective incorporation of BC into the subsoil by peloturbation in combination with larger input. Thixotropic Andosols and Alisols likely bound BC in mineral-organic complexes. However, we could not find a direct correlation between Al and Fe oxide concentrations and BC. A positive effect of paddy management on BC stocks was found only in unburned soil, likely due to a masking by high input of burned waste and crop residue at the other non-paddy sites under study.

We detected aromatic N in rice char and some soils, but found no hint of BN accumulation via burned crop residues. Our observations instead suggested that aromatic N in soil forms from other sources, such as during ageing of organic N.

Overall, we conclude that in these systems differences in BC input and physical effects (such as soil texture, dense plow layers, and cementation by Fe oxides) control BC storage along soil-depth profiles more than do chemical processes.

V.
SYNOPSIS

1 Introduction

The economical and resource-saving production of rice is crucial for the nutrition of mankind. One major problem in paddy soils is the low N use efficiency (Cassman et al. 1996b; Kögel-Knabner et al. 2010a) caused by high emissions of NH_3, N_2, and N_2O, by NO_3^- leaching and N sequestration processes in soil (Cassman et al. 1996b; Geisseler et al. 2010a, Roth et al. 2011, Jiang et al. 2013). Promising research resulted from a 2000 years paddy soil chronosequence suggesting that paddy soils may sequester significant amounts of N in microbial residues and aged amino acids (Roth et al. 2011). In addition, an accumulation of charred organic matter with yet unknown contribution to total N amount was observed (Kögel-Knabner et al. 2010; Lehndorff et al. 2014). However, it remained unclear to which degree such processes also hold true for paddy soils formed from different parent materials and in different countries. Therefore, I studied the influence of paddy management and different underlaying parent material on soil organic nitrogen (SON) and thermally altered C and N sequestration. Paddy and non-paddy cropped soils were sampled in three different climate zones (tropical, subtropical and temperate). The soil profiles comprised three replicates of Andosols, Alisols and Vertisols from Java (Indonesia), Alisols (China), and a Fluvisol and Gleysol (Northern Italy).

As mentioned in the General Introduction (see chapter I), I formulated three hypotheses:

2 Summary of the results

i. Does paddy management lead to N sequestration in peptide bonds?

Long-term paddy management promotes nitrogen sequestration, but it is unknown to what extent the properties of the parent material control the management-induced nitrogen sequestration in amino acids. I hypothesized that paddy management effects on the storage of N in amino acids (AA-N) relate to the mineral assembly. Hence, I determined contents and chirality (as marker for ageing) of peptide-bound amino acids in paddy soils from different major reference soil groups (Vertisols, Andosols, Alisols in Indonesia, Alisols in China, and a Gleysol/Fluvisol in Italy). Adjacent, non-paddy soils served as references. Representative top- and subsoil samples were pre-extracted with dithionite–citrate–bicarbonate (DCB) to better

understand the role of reactive oxide phases in AA-N storage, origin, and composition. The results showed that topsoil N- and AA-N stocks were significantly larger in paddy-managed Andosols and Chinese Alisols than in their non-paddy counterparts. In other soils, however, paddy management did not cause higher stocks of N- and AA-N, possibly because N-fixing intercrops masked the paddy management effects on N sequestration processes. Among the different major reference soil groups, AA-N stocks were largest in Andosols, followed by Alisols and Gleysol/Fluvisol pair, and lowest in Vertisols. The N storage in amino acid went along with elevated D-contents of bacteria-derived alanine and glutamic acid, as well as with increasing stocks of DCB-extractable oxides. D-amino acids, likely formed by racemization processes, did not vary systematically between paddy and non-paddy managed soils. My data suggests that the presence of oxides increase the N sequestration in amino acids after microbial N transformations.

ii. Do bacteria or fungi promote high microbial N sequestration in paddy soils?

Paddy management promotes nitrogen sequestration in microbial residues, but the mechanism and influencing factors are not fully understood. Paddy management inhibited the microbial decomposition of organic matter. Here, I assume that the slower build-up of microbial residues became easier protected from further decay by mineral-organo interactions, which were promoted by different redox phases. As a result, paddy soils should sequester more nitrogen in microbial residues than other cropping systems. I thus hypothesized that paddy management led to higher proportion of bacteria (vs. fungi), related to non-paddy managed soils due to better management adaptability (like waterlogging, puddling and intensive fertilization). Further, I hypothesized that the mineral assembly of the parent material is crucial for long-term N sequestration in microbially decomposed soil organic nitrogen via mineral-organo interactions. To test these hypotheses, I determined carbon and nitrogen stocks, $^{14}/^{15}$N isotopes, amino sugar stocks, and calculated the microbial residue stocks in paddy and adjacent non-paddy top- and subsoils from different major reference soil groups (Vertisols, Andosols, Alisols in Indonesia, Alisols in China, and a Gleysol/Fluvisol in Italy). For soil characterization, I determined pH value, soil texture, and DCB-extractable oxides.

Surprisingly, I did not find consistently larger organic carbon-, nitrogen-, and microbial residue stocks in paddy soils compared to adjacent non-paddy soils. Further, I could not confirm that paddy management led to higher proportions of bacteria (vs. fungi), related to non-paddy-managed soils. However, I found indices for a different response of bacterial and

fungal residue stocks to applied anthropogenic management, e.g., N-fixing intercrops enhanced N sequestration in bacterial residues.

Independent from management, an increase of total N was correlated to an increase of microbial residue stocks in all soils under study. Soils with high stocks of DCB-extractable pedogenic oxides obtained larger microbial residue stocks, supporting my overarching assumption that the mineral assemblage of the parent soil significantly contributed to the storage of microbe-derived N.

iii. To which extent can N sequestration in paddy soils be assigned to input of charred organic matter?

Laboratory studies indicated that the turnover of soil black carbon (BC) also depends on soil mineral assembly. I hypothesized, therefore, that also the overall storage of BC and black nitrogen (BN) in soil relates to the abundance of reactive mineral phases, such as of Al, weak crystalline Fe oxides, and clay. Burning of crop residues and frequent flooding of paddy soil can lead to significant accumulation of BC, thus contributing to long-term C sequestration. In parallel, BN should accumulate in soil. Samples were analyzed for soil organic carbon (SOC) and BC, the latter by oxidation to benzene polycarboxylic acids (BPCAs). Abundance of BN (as aromatic N) was determined via XPS analysis for representative topsoil horizons.

BC contents relative to SOC were surprisingly constant in each soil profile, suggesting that BC co-accumulated with SOC in all soils. The overall stocks of BC, however, differed from SOC stocks and were mostly soil-specific. Vertisols contained largest BC stocks (17-19 t ha^{-1} in non-paddy and paddy fields), followed by Andosols and Alisols (6-10 t BC ha^{-1} under paddy management; 3-8 t ha^{-1} under non-paddy management); the Gleysol and Fluvisol had lowest BC stocks (3-4 t ha^{-1}). In pre-tests, aromatic N proportions increased to > 50% of total N after combustion of rice straw. However, aromatic N was barely, or not detectable in studied soils, and there was no correlation to BC. I conclude that burned crop residues were not a major source of aromatic N in soil. BC and aromatic N showed no distinct relations to soil properties, such as the abundance of clay-sized minerals, and Al and Fe oxides. Differences in BC stocks between the soils were most pronounced in the subsoils, likely caused by physical processes, such as swelling and shrinking of clays and/or translocation by leaching.

3 Synthesis

General discussion

Surprisingly, I did not find consistently higher organic carbon-, nitrogen-, and microbial residue stocks in paddy soils compared to adjacent non-paddy soils formed on different parent materials. Indeed, my data suggested that rather the parent material controls microbial residue stocks and that the applied paddy management played a minor role for N sequestration in microbial residues under long-term cultivation. However, my sampling design of five paddy and non-paddy soils formed on different parent materials represented only a snapshot in a long-term history of paddy and non-paddy management. Yet, already from these few profile studies I may refuse the hypothesis that paddy management always leads to elevated N contents and microbial residue stocks in all paddy soils, because the content of oxides in soils overruled the effect of paddy management, i.e., frequent flooding, on total N and microbial residue N storage. To verify my new findings, I performed an additional experiment to understand the processes involved in N cycling in paddy soils. Therefore, I submerged aerobic agricultural topsoil (non-paddy) and cropped them for one season with rice. I assumed that the submergence of a non-paddy topsoil would influence the short-term fertilizer N cycling and sequestration in microbial residues.

Microbial N sequestration in paddy soils

To be able to elucidate differences in microbial N sequestration in paddy soils compared to non-paddy soils, I performed a ^{15}N-labeling greenhouse experiment at the "Helmholtz Zentrum München". It should shed light on short-time effects of paddy management on N sequestration in microbial residues under similar soil conditions to minimize the influence of different soil properties. I chose the topsoils (0-15 cm) of the Italian paddy (Gleysol) and non-paddy (Fluvisol) for this study, because of unlimited supply of soil from this site. The experimental setup comprised three variants (n=4): ancient paddy soil cropped with rice (P-R) as control for continuous paddy cultivation, non-paddy soil cropped for the first time with rice (NP-R) and the same non-paddy soil cropped with maize (NP-M) as control for continuous non-paddy cultivation. All variants were distinguished between ^{15}N-fertilized and non-labelled-fertilized, but special interest referred to the recently submerged non-paddy soil cropped with rice (NP-R). From literature it is well known that with the submergence of rice fields the amount of algaes, fungi and actinomycetes decreases, while bacteria species dominate (Bossio and Scow, 1998; Kyuma, 2004; Nakamura et al., 2003; Said-Pullicino et al.,

2014; Tian et al., 2013). Additionally, more obligate anaerobes (e.g. sulfate reducers), facultative anaerobes (e.g. denitrifiers) and archaea occur in paddy soils compared to non-flooded soils (Bai et al., 2000; Kyuma, 2004). Therefore, I assumed that the topsoil of the recently submerged non-paddy soil cropped with rice will show enhanced N sequestration in microbial residues compared to the non-paddy soils with maize (NP-M).

The experiments were carried out for one growing season, and samples of soil were taken at the beginning (days 0), before flowering/tillering (day 55) and close to harvest/flowering (day 90) (for detailed information of the location and greenhouse experiment set up see Fig.IX 1 and Fig.IX 2 in Appendix D). The microbial N sequestration was determined by estimation of microbial residues via amino sugar analysis and ^{15}N enrichment in selected amino sugars (glucosamine and muramic acid), which served as biomarkers for fungi and bacteria. For estimation of applied ^{15}N fertilizer in the three variants, I determined the ^{15}N recovery of the bulk soil.

At the beginning of the greenhouse experiment, the ancient paddy soil obtained slightly lower microbial residue contents compared to the non-paddy soil (Fig.IX 3 in Appendix D). During the 90 days, the content of microbial residues did not change significantly over the time in each variant ($p > 0.1$). Furthermore, I could not find any significant differences between the three variants ($p > 0.1$) (for detailed results see Appendix D, chapter 3. Results).

The glucosamine (Glu) to muramic acid (MurA) ratio of the ancient paddy soil (P-R) dropped due to increased concentrations of muramic acid. In the non-paddy soil cropped with rice (NP-R), the Glu to MurA ratio remained constant over time (Fig.V 1). In the non-paddy soil cropped with maize (NP-M), the Glu to MurA ratio increased, due to the increased concentration of glucosamine and decreased concentrations of muramic acid over time.

The ancient paddy soil and the recently submerged non-paddy soil cropped with rice (NP-R) did not differ from each other significantly ($p = 0.163$) but were significantly different from the soil cropped with maize (NP-M) ($p < 0.001$).

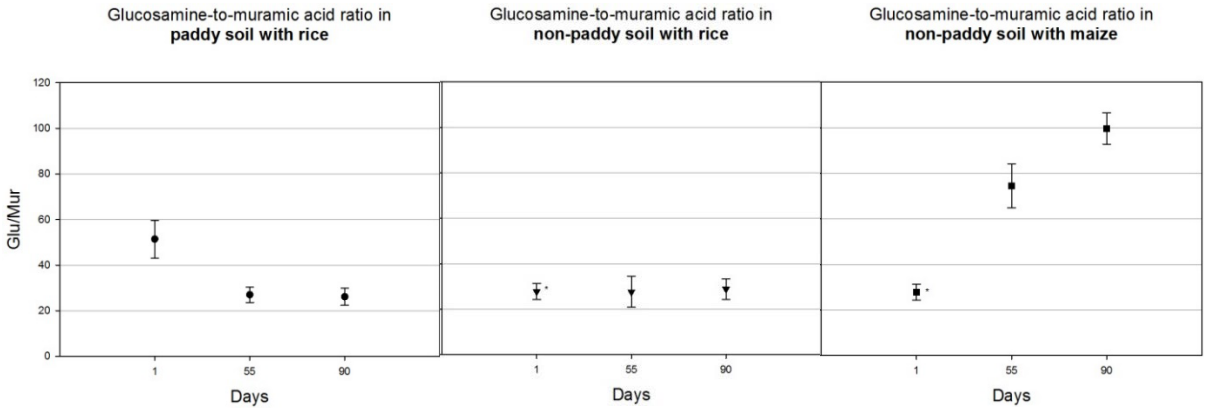

Fig.V 1: Glucosamine (Glu)-to-muramic acid (Mur) ratio in the paddy soil with rice, non-paddy soil planted with rice and non-paddy soil planted with maize at day 1 (pre-seeding), day 55 (rice tillering) and day 90 (rice flowering). Asterisks indicate similarity (at day 1 there was no differentiation between non-paddy soils, because planting of rice and maize did not occur yet). The error bars represent the standard deviations of the field replicates (n = 4).

^{15}N recovery decreased in the direction non-paddy soil with maize > non-paddy soil cropped with rice = paddy soil with rice ($p<0.05$ for the comparisons of NP-M with the other two soils; $p= 0.55$ for the comparison of NP-R with P-R, for detailed results see Appendix D, chapter 3. Results). Similar low N fertilizer recoveries in paddy systems have also been reported by other researchers (e.g. Zhao et al., 2009; Dong et al., 2012, Zhang et al., 2012, Cao and Yin, 2015), who attributed these elevated N losses to submergence as a primary factor for NH_3 volatilization, runoff and leaching, in addition to denitrification losses as indicated above.

The low recoveries of fertilizer N suggested also that microbial sequestration processes were fairly ineffective in retaining the fertilizer ^{15}N in soil (Fig.IX 4 in Appendix D). Nevertheless, incorporation of ^{15}N fertilizer into microbial residues (determined in glucosamine and muramic acid) were detectable and highest in the paddy soil cropped with rice (P-R), followed by the non-paddy soil with rice (NP-R) and lowest in the non-paddy soil with maize (NP-M), despite the latter retained the highest overall amount of fertilizer N. The N fertilizer incorporation into amino sugars was assessed by atom percentage excess (APE).

Contrary to my expectations, there was no systematic shift from bacterial N residues to fungal N residues, only the variant with the long-term paddy soil with rice (P-R) showed higher ^{15}N fertilizer incorporation in bacterial and fungal residues, compared to the two other variants (Fig.V 2). For detailed analytic method and calculation description see Appendix D, chapter 2. Materials and Methods.

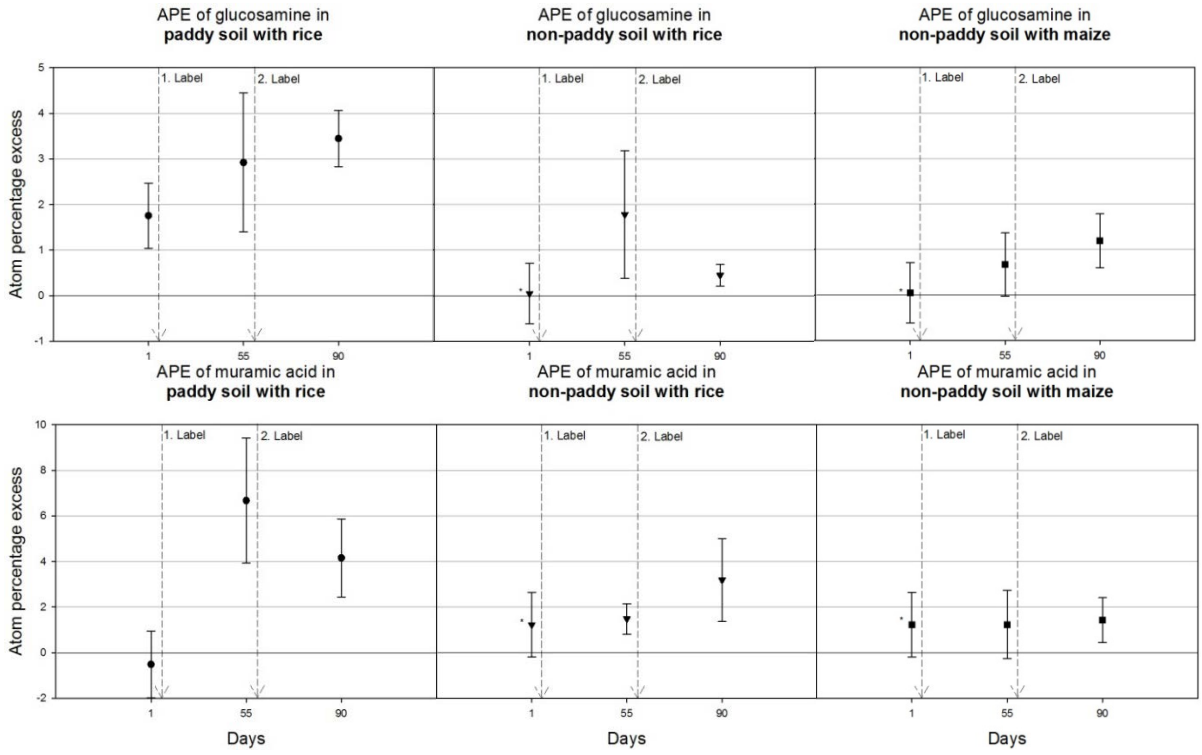

Fig.V 2: ^{15}N fertilizer recovery expressed as atom percentage excess (APE) of glucosamine (biomarker for fungal residues) and muramic acid (biomarker for bacterial residues) in the paddy soil with rice, non-paddy soil cultivated with rice and non-paddy soil with maize at day 1 (pre-seeding; non-labeled), day 55 (tillering; 55 days after first labeling, with 127.575 mg N urea per pot) and day 90 (flowering; 35 days after second labeling, with 72.9 mg N urea per pot). The error bars represent standard deviations of the greenhouse pot replicates (n=4).

In summary, I could not find a higher N sequestration in microbial residues after one season of rice cropping on a non-paddy soil.

However, my data showed that the N cycle is immediately influenced by paddy management, as evidenced by low overall ^{15}N recovery, however, microbial N transformation rates were slow. It is thus unlikely that one specific cropping season may affect the amount of microbially sequestered N significantly. Hence, the detected differences among our sites more likely reflect true long-term, repeated cycling of microbial residues, which according to recent evidence exhibit a mean residence time of up to 8 years (Derrien and Amelung, 2011; Fuhrmann et al., 2018).

However, my data showed that recently introduced paddy management on a non-paddy soil decreased the ^{15}N recovery of applied N fertilizer in bulk soil immediately compared to the non-paddy soil with maize (Fig.IX 4 in Appendix D). Several studies reported similar low N fertilizer recoveries in paddy systems of 10 - 24 % and 23 - 29 % in the wheat season (Cao and Yin, 2015; Dong et al., 2012; Zhao et al., 2009; Zhang et al., 2012) and higher N fertilizer recoveries in non-paddy systems (e.g., 13 – 45 % in wheat season and wheat-maize rotation (Zhao et al., 2009, Ju et al., 2007). They determined the submergence as a primary factor,

which rapidly promotes NH_3 volatilization, runoff and leaching and assumed this as the major factors for N losses in paddy soils. Particularly, NH_3 can be formed of urea, so that in flooded soils NH_3 volatilization achieved highest peaks after three days of urea application (Shang et al., 2014). These studies underline our findings that N fertilizer recovery in soil also quickly responds to management changes. Hence, I assume that the low N fertilizer recovery in the paddy and non-paddy soil cropped with rice could be caused by higher NH_3 volatilization, which was induced by the submergence.

With regard to the microbial residues, the non-paddy cropped with rice differs to the non-paddy with maize, which gives support to the question that newly converted soils are more vulnerable to changes in microbial N sequestration than others. Apparently, the recently applied paddy management inhibited a shift to more fungal microorganisms (as observed in the non-paddy with maize) so that larger portions of bacterial residues prevailed. This agrees with previous studies, which reported that bacteria dominate fungi in paddy (or rather submerged) soils (Bossio and Scow, 1998; Drenovsky et al., 2004; Nakamura et al., 2003; Said-Pullicino et al., 2014; Tian et al., 2013; Unger et al., 2009). Also Roth et al. (2011) found a faster adaption of bacteria than of fungi to submergence. This supports my assumption of a decreasing fungal biomass and a relative accumulation of bacterial biomass in the non-paddy soil with rice. Hence, I assume microbial N sequestration in paddy soils is species-specific, even if effects on fungal and bacterial residue dynamics are in part compensated so that the overall amount of microbially sequestered N was not affected. However, in summary I could not find significant differences in microbial N sequestration between paddy and non-paddy soils, which supports my hypothesis that the mineral assemblage plays a major role in microbial N sequestration.

Mineral controls on the sequestration of N in fungal and bacterial residues

Any storage of N in microbial residues is controlled by the balance between microbial transformation rates (N loss, catabolic pathways) and microbial sequestration of N (anabolic pathways; Liang et al., 2017). Indeed, low fertilizer recovery after land submergence and several earlier studies showed that there are significant denitrification losses in paddy soils (Cassman et al. 1996, Geisseler et al. 2010a). However, microorganisms also contribute to the low N use efficiency by immobilizing N in their residues (Cucu et al., 2014; Said-Pullicino et al., 2014; Roth et al., 2013; Ding et al., 2011a). These N sequestration processes operate at a scale of decades (Roth et al. 2011, Said-Pullicino et al.; 2014, see also results from the greenhouse study outlined above). They do thus interlink with other process of SOM

stabilization, such as sorption at or encrustation within soil particles (Christensen, 1992: Ladd et al., 1993). Lützow et al. (2008) thus concluded that stabilization of SOM by interaction with minerals is of increasing importance in the long-term cycling of SOM. With regard to SON, amino acids and amino sugars can be bound to minerals by cation exchange reactions of their amino group or by ligand exchange with metal hydroxides by their carboxyl group (Cheshire et al. 2000).

The suggestion that different parent material contributes also to different storage of SON in tropical soils was, e.g., also investigated by Moritz et al. (2009). They reported for tropical forests that the sedimentary substrates preserved higher amounts of amino sugars than metamorphic, ultrabasic substrates and linked this to changes in iron oxide crystallinity in the subsoil. Another study was published for a chronosequence of aerated, volcanic soils in Hawaii (Mikutta et al. 2010). They investigated amino sugars, amino acids and ^{14}C content of mineral-bound SON and could show a strong initial association of acidic amino acids to metal (hydr-)-oxides, organic precipitates and variable-charge minerals. They conclude that the mineral-associated N in the crystalline topsoils seemed to be actively involved in N cycling.

In paddy soils, Fe oxides undergo reductive dissolution under anoxic, and re-precipitation under oxic conditions. The re-formation of poorly crystalline Fe in oxic phases may additionally contribute to SON accumulation due to co-precipitation of organic matter (Eusterhues et al., 2011, Chen et al., 2014). As a result, paddy soils with high contents of pedogenic oxides sequester more nitrogen in microbial residues compared to other cropping systems. These findings are in line with my data. The volcanic parent material, on which Andosols in my study were formed, could thus support a sequestration of microbial N due to elevated contents of reactive, short range-ordered Fe oxides and allophane-imogolite-type phases (Winkler et al. 2016). Indeed, I found largest Nt stocks in Andosol topsoils. What is more, also microbial residue N was enriched though at similar magnitude as in the Javenese Alisols, which are also rich in pedogenic oxides (Winkler et al., 2016). It seemed thus reasonable to conclude that pedogenic oxides play a major role for N sequestration in paddy soils. The other soils in my study contained lower pedogenic oxide contents and I did find an enhanced N sequestration in the paddy soils compared to their non-paddy counterparts. Furthermore, I hypothesized that paddy management led to higher proportion of bacteria (vs. fungi), related to non-paddy managed soils due to better management adaptability (like waterlogging, puddling and intensive fertilization). I could not confirm this hypothesis, neither in the field study nor in the greenhouse study.

From literature, fungi usually dominate in acidic soils with low amounts of available nutrients, recalcitrant organic matter, and high C/N ratios (Blagodatskaya und Anderson 1998; Holland und Coleman 1987). The populations of fungal hyphae grow slowly, but are more resistant against further microbial decomposition than bacterial polymers (Guggenberger et al. 1999; Webley and Jones 1971). In nutrient-rich soils, in contrast, the bacterial community was found to dominate due to their ability for fast cycling of nutrients and accompanied rapid growth of communities. Additionally, they provide a rather quick response to environmental changes (Hayat et al., 2010; Ingwersen et al. 2008). It is know, that the microbial community is sensitive to anthropogenic management. Hence, I assume that the diverse crop management on my field study may overwrite higher proportions of bacterial residues in paddy soils. For instance, the Andosol-derived paddies had Chinese cabbage as intercrop. Usually only the upper leaves were harvested with no submergence of the soil and these results in a high remain of organic matter (Hill 1990). A comparable organic matter input appeared at the non-paddy sites with the cultivation of cassava. Liang et al. (2007) found that high plant material in put significantly increased microbial activity and residues. I assume that the intercrop on the paddy site and management on the non-paddy site overwrites effects of paddy management.

At the Javanese Alisol site, the farming was less intensive. The crop rotation was diverse (cabbage, cassavas and bananas), plant residue input was higher and fertilizer application was lower. Dick (1992) mentioned that manure, and plant diversity is more important in maintaining soil microbial activity than conventional tillage in monoculture systems. The non-paddy fields were fertilized with manure and showed higher bacterial residue stocks. Additionally, the non-paddy fields were surrounded with legume trees. Therefore, I assume that the bacterial community was more prominent, which resulted in higher $\delta^{15}N$ values at the Javanese Alisol site.

At the Vertisol site, the intensive land-use and the input of fresh organic matter likely promoted bacterial N cycling as observed in another study (Joergensen und Wichern 2008), however, the Vertisols still obtained the lowest microbial residue stocks. Monoculture systems, like on the Vertisol sites, mainly lead to a decrease of diversity in the microbial community (Dick 1992). Also an intensive use of mineral fertilizer can reduce the microbial community (Yoneyama et al., 1990). Intensive tillage decreases the fungal community, due to their preference of continuous soil structure stability to build their complex hyphae in macroaggregates (Chantigny et al. 1997; Simpson et al. 2004). At the studied Vertisol sites the sugar cane fields and the paddy fields were deeply ploughed and both temporarily

submerged. I assume that ploughing, the Vertisol-inert self mulching, and the low content of pedogenic oxides hinder microbial N sequestration. Although the rates of bacterial turnover are higher in the Vertisols, missing stabilization mechanisms affected sequestration of bacterial N.

The Gleysol/Fluvisol pair differed regarding their agricultural management to the other studied soils, as there was no puddling, only one harvest per year and a long fallow period. The hyphae net of the fungi community grows slowly and prefers long fallow periods and/or low tillage because of slow growth in macroaggregates (Chantigny et al., 1997, Guggenberger et al., 1999). Furthermore, fungi need less fresh organic matter input due to their better decomposition efficiency for recalcitrant organic compounds (Joergensen und Wichern 2008). Therefore, the $\delta^{15}N$ values and AZ-N stocks were slightly lower at the Italian site compared with the other sites, which support my assumption that fungal residues were dominant in Italian site.

In summary, I found indices that the microbial community responded to fertilization, plant residue input, tillage and crop rotation. However, this was not the focus of my study and to verify these ideas, further research is necessary.

4 Outlook

In this study, I was able to elucidate that the parent material plays a major role for long-term N sequestration in paddy soils. My data suggests that the presence of pedogenic oxides increase the N sequestration after microbial N transformation. To elucidate to role of pedogenic oxides and assumed accompanied increase of SON aging, dating of isolated amino acids or, even better, of isolated amino sugars, would be necessary. As described in the first chapter of this study, microbially transformed amino acids were enriched in Andosols but hardly accumulated in Vertisols. As oxide removal went along with a loss of amino acids including those typical for peptidoglycane, I infer that oxidic mineral phases controlled long-term organic N cycles irrespective from current land-use. Elucidating the net rates of microbial N sequestration, e.g., by using compound-specific stable isotope analyses and radiocarbon dating, might now warrant further attention. A challenging task will be the dating of the age markers itself, i.e., of specific D-amino acids as a clue for racemization rates.

VI.

APPENDIX A

Supporting information to Chapter II

Tab.VI 1: D/L ratios of alanine, glutamic acid, aspartic acid and lysine; n= number of field replicates, *= mean of one field replicate and two interpolated values.

		Paddies					Non Paddies				
		soil	D/L ratios of				soil	D/L ratios of			
site	n	depth [cm]	Alanine	Glutamic acid	Apartic acid	Lysine	depth [cm]	Alanine	Glutamic acid	Apartic acid	Lysine
Andosols (Java)	3	0-8	0.17 ± 0.04	0.12 ± 0.01	0.19 ± 0.01	0.04 ± 0.00	0-7	0.19 ± 0.03	0.15 ± 0.03	0.37 ± 0.04	0.02 ± 0.01
	*	8-22	0.18 ± 0.04	0.12 ± 0.01	0.21 ± 0.01	0.04 ± 0.01	7-16	0.22 ± 0.03	0.15 ± 0.03	0.53 ± 0.08	0.02 ± 0.01
	3	22-29	0.19 ± 0.04	0.14 ± 0.01	0.24 ± 0.02	0.05 ± 0.02	16-28	0.27 ± 0.08	0.17 ± 0.04	0.86 ± 0.17	0.03 ± 0.02
	*	29-35	0.23 ± 0.03	0.15 ± 0.03	0.41 ± 0.14	0.05 ± 0.02	28-56	0.29 ± 0.07	0.16 ± 0.01	0.99 ± 0.22	0.02 ± 0.01
	3	35-50	0.32 ± 0.07	0.15 ± 0.01	0.70 ± 0.25	0.04 ± 0.01	56-78	0.23 ± 0.01	0.18 ± 0.03	1.06 ± 0.18	0.04 ± 0.01
	*	50-75	0.26 ± 0.01	0.17	0.83 ± 0.07	0.04 ± 0.00	78-100+	0.37 ± 0.00	0.14	1.12 ± 0.00	0.02
	1	75-105+	0.41	0.15	0.91	0.03					
Alisols (Java)	3	0-7	0.16 ± 0.03	0.13 ± 0.01	0.08 ± 0.01	0.05 ± 0.01	0-7	0.19 ± 0.03	0.15 ± 0.02	0.09 ± 0.00	0.03 ± 0.02
	*	7-15	0.21 ± 0.04	0.14 ± 0.01	0.11 ± 0.02	0.05 ± 0.02	7-14	0.21 ± 0.01	0.17 ± 0.01	0.10 ± 0.01	0.05 ± 0.00
	3	15-20	0.23 ± 0.05	0.16 ± 0.02	0.12 ± 0.03	0.06 ± 0.02	14-22	0.21 ± 0.01	0.20 ± 0.02	0.12 ± 0.02	0.08 ± 0.04
	*	20-55	0.29 ± 0.06	0.15 ± 0.02	0.17 ± 0.02	0.07 ± 0.01	22-46	0.24 ± 0.02	0.20 ± 0.03	0.15 ± 0.02	0.07 ± 0.03
	3	55-72	0.30 ± 0.05	0.17 ± 0.03	0.23 ± 0.02	0.08 ± 0.05	46-63	0.29 ± 0.07	0.19 ± 0.05	0.17 ± 0.03	0.07 ± 0.02
	1	72-90+	0.32	0.15	0.22	0.11	63-83	0.26	0.19	0.16	0.07
	1						83-100+	0.39	0.15	0.18	0.08
Alisols (China)	3	0-8	0.09 ± 0.01	0.07 ± 0.00	0.05 ± 0.00	0.03 ± 0.01	0-12	0.13 ± 0.01	0.13 ± 0.02	0.06 ± 0.01	0.03 ± 0.01
	*	8-15	0.11 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	12-22	0.17 ± 0.02	0.15 ± 0.01	0.08 ± 0.01	0.02 ± 0.01
	3	15-20	0.11 ± 0.02	0.09 ± 0.00	0.07 ± 0.00	0.05 ± 0.01	22-33	0.18 ± 0.01	0.20 ± 0.02	0.11 ± 0.02	0.04 ± 0.01
	*	20-28	0.15 ± 0.01	0.10 ± 0.02	0.10 ± 0.00	0.05 ± 0.02	33-56	0.20 ± 0.02	0.20 ± 0.02	0.13 ± 0.03	0.04 ± 0.01
	3	28-42	0.21 ± 0.03	0.13 ± 0.01	0.14 ± 0.01	0.06 ± 0.02	56-80	0.19 ± 0.01	0.19 ± 0.03	0.16 ± 0.05	0.04 ± 0.02
	*	42-60	0.22 ± 0.03	0.16 ± 0.03	0.20 ± 0.03	0.09 ± 0.01	80-100+	0.21 ± 0.01	0.20 ± 0.02	0.16 ± 0.02	0.06 ± 0.04
	1	60-95+	0.32	0.18 0.07	0.26	0.10					
Fluvisol	1	0-13	0.11	0.09	0.07	0.09	0-15	0.16	0.14	0.07	0.07
Gleysol	1	13-25	0.12	0.08	0.06	0.06	15-30	0.16	0.13	0.07	0.06
(Italy)	1	25-34	0.14	0.1	0.08	0.07	30-70	0.19	0.19	0.14	0.09
	1	34-43	0.18	0.15	0.12	0.13	70-85+	0.17	0.1	0.62	0.09
	1	43-53	0.22	0.18	0.16	0.15					
	1	53-80	0.23	0.19	0.16	0.12					
	1	80-90	0.19	0.2	0.15	0.13					
	1	90-110+	0.20	0.21	0.18	0.13					
Verisols (Java)	3	0-8	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0-10	0.12 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.02 ± 0.01
	*	8-20	0.15	0.09	0.09	0.01	10-23	0.16	0.11	0.08	0.00
	3	20-27	0.14 ± 0.06	0.11 ± 0.00	0.11 ± 0.00	0.05 ± 0.02	23-29	0.09 ± 0.03	0.14 ± 0.04	0.12 ± 0.05	0.02 ± 0.02
	*	27-47	0.18	0.08	0.12	0.01	29-55	0.15	0.09	0.11	0.02
	3	47-90	0.16 ± 0.06	0.14 ± 0.04	0.16 ± 0.04	0.08 ± 0.04	55-100	0.13 ± 0.04	0.12 ± 0.04	0.13 ± 0.02	0.04 ± 0.01
	1	90-115+	0.16	0.09	0.13	0.01	100-120+	0.16	0.08	0.13	0.02

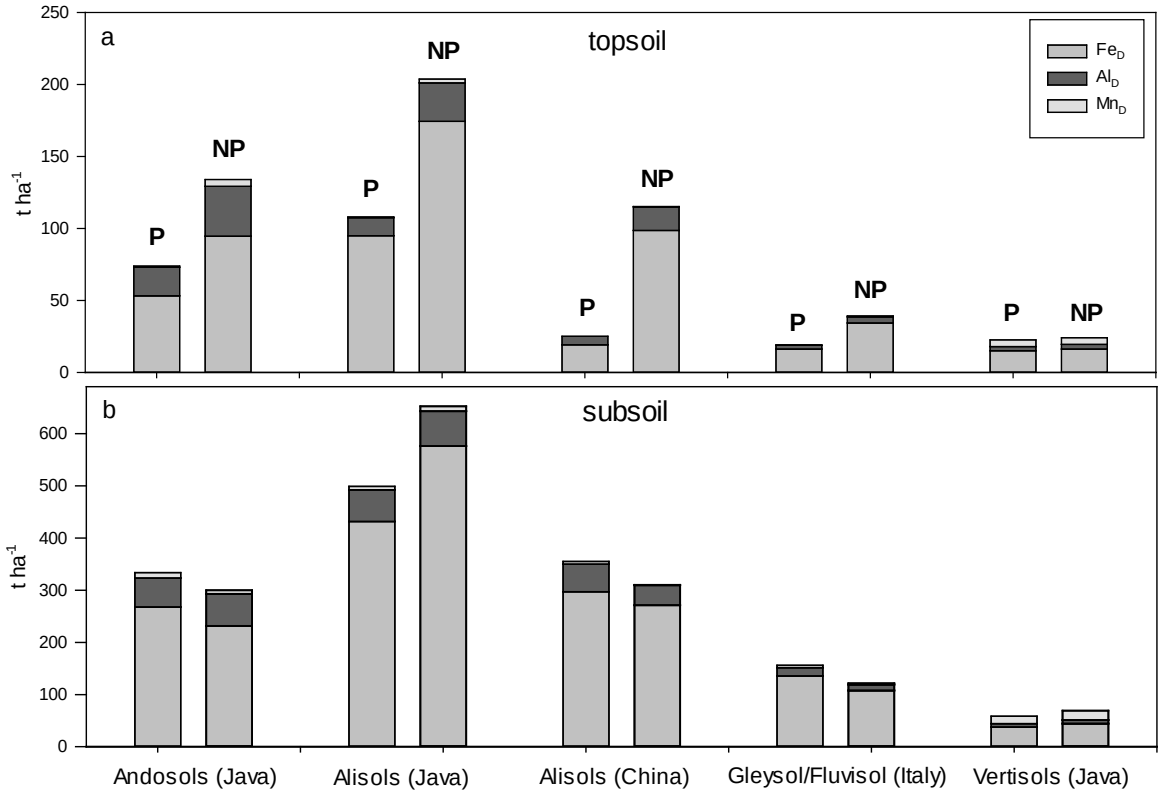

Fig.VI 1: (a,b) Dithionite-extractable Fe_D, Al_D and Mn_D stocks in paddy (P) and non-paddy (NP) in (a) top- and (b)subsoils of five major reference soil groups (3 field replicates, except for Italian site); data taken from Winkler et al., 2016).

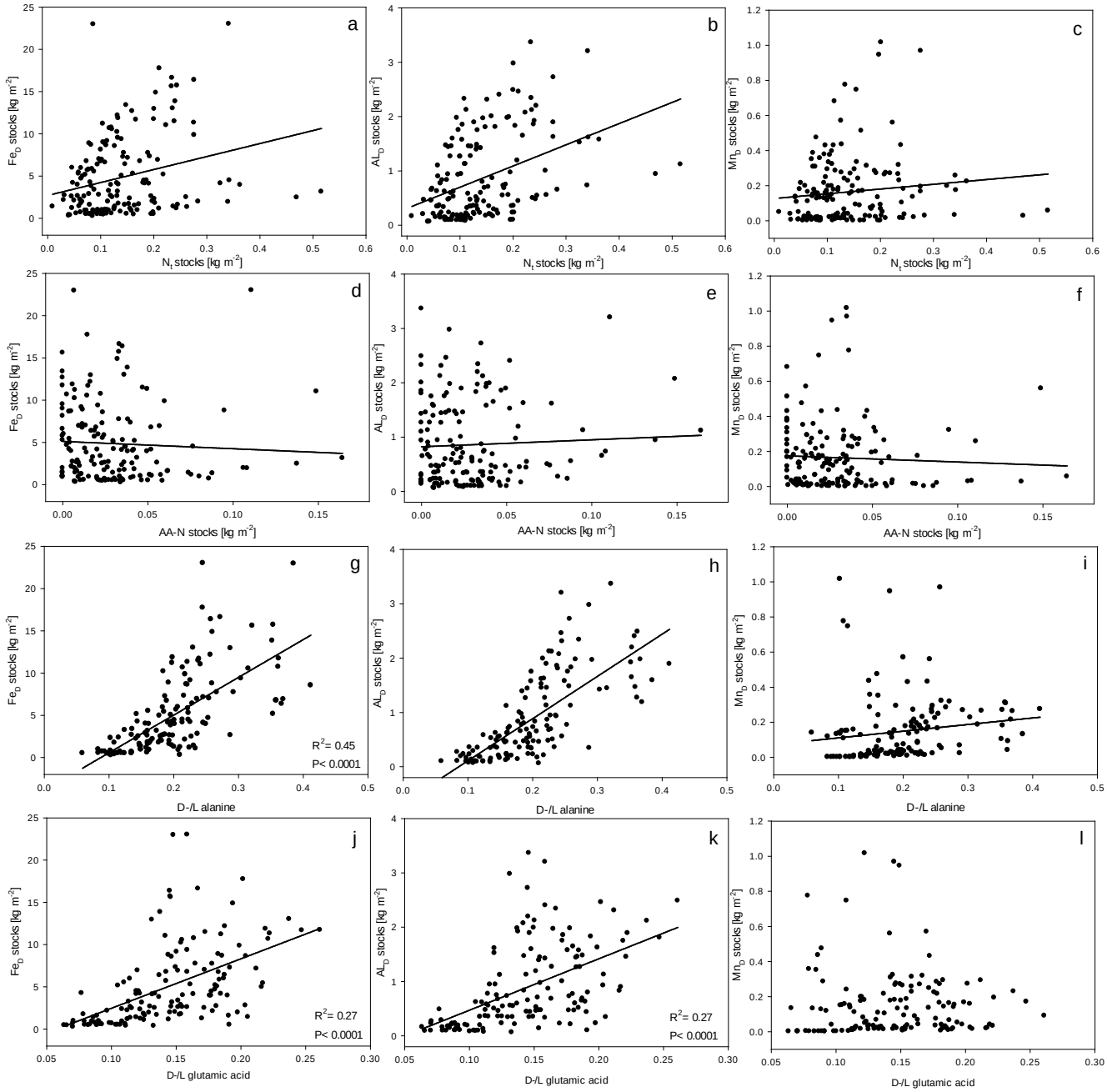

Fig.VI 2: Relationships between dithionite extractable Fe_D, Al_D and Mn_D stocks and the stocks of N_t AA-N, and the D/L ratio of alanine and glutamic acid.

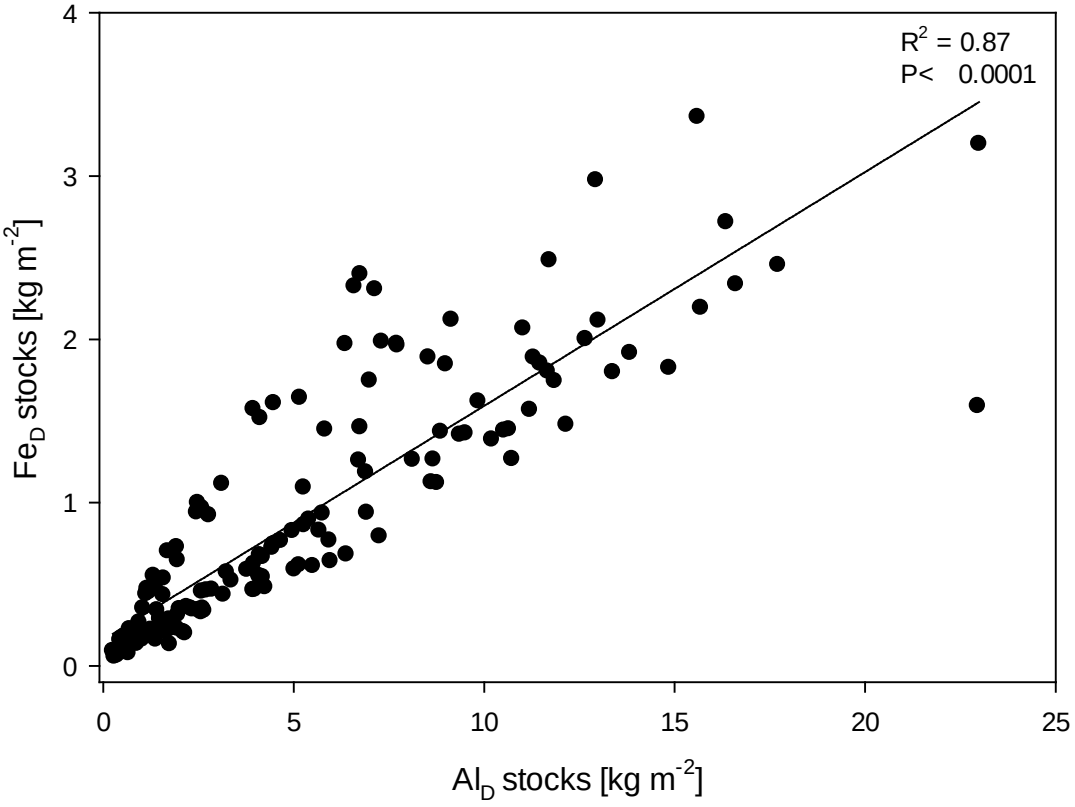

Fig.VI 3: Correlation between dithionite-extractable Fe_D and Al_D indicates atomic substitution exchange of Fe for Al in soil oxides.

VII.

APPENDIX B

Supporting information to Chapter III

Tab.VII 1: Background information to the study sites (obtained from field observation, soil classification and interviews with farmers)

Soil type	Country	Texture	Precipitation [mm] / climate	Crop rotation	Fertilizer per year	Tillage form	Yield per year	Herbcides/fungicides	Elevation [m a.s.l.]	Coordinates
Andosol-derived paddy Escalic Hydragrick Anthrosol (Dystric)	Indonesia (Java) Sukabumi	Silt-clay loam	2000-4400 / tropical (25°C)	*Oryza sativa* (rice), *Oryza sativa* , *Brassica rapa chinensis* (cabbage)	Urea, NPK, SP-36 (superphosphate, 36%) and partly manure	Puddling with hand tractor	5 -6 t ha^{-1}	None	870-871	S 06°52.802‘/ E 106°56.457
Silandic **Andosol** (Distric, Thixotropic)				*Manihot esculenta* (cassava), *Zea mays* (maize)	Manure, urea and NPK*	Hoe	Unknown	None	969-981	S 06°52.029‘/ E 106°56.725‘
Alisol-derived paddy Escalic Hydragrick Anthrosol (Dystric, Clayic)	Indonesia (Java) Jasinga	Silt loam	2500-3500 / tropical (27°C)	*Oryza sativa* (rice), *Oryza sativa* , *Zea mays* (maize)	300 kg ha^{-1} urea, 200 kg ha^{-1} NPK, SP-36 (superphosphate, 36%) and manure	Puddeling with buffallos	3 t ha^{-1}	None	238-248	S 06°32.205‘/ E 106°31.062‘
Hyperalic **Alisol** (Abruptic, Humic, Clayic, Chromic)				*Manihot esculenta* (cassava), *Musa spec.* (banana), *Leucaena leucocephala* (mimosa)	Only manure	Hoe	Unknown	None	236-238	S 06°32.158‘/ E 106°31.030‘
Vertisol-derived paddy Hydragrick Anthrosol (Eutric, Clayic)	Indonesia (Java) Ngawi	Clay-silt clay	2000-3000 / tropical (27°C)	*Oryza sativa* (rice), *Oryza sativa* , *Nicotiana spec.* (tobacco)	50 kg ha^{-1} NPK*, 50 kg ha^{-1} ammonium sulfate, 25 kg ha^{-1} urea, 25 kg ha^{-1} TSP (triple superphosphate, 46%) and 25 kg ha^{-1} SP-36 (superphosphate, 36%)	Puddling with hand tractor	Unknown	Unknown	77-78	S 07°26.878‘/ E 111°36.599‘
Pisocalcic Mollic Grumic **Vertisol** (Humic, Hypereutric, Pellic)				*Saccharum officinarum* (sugar cane)	1000kg ha^{-1} green manure, 200 kg ha^{-1} ammonium sulfate, 600 kg ha^{-1} NPK*	Hand tractor	10-15 t ha^{-1}	Cidamin	78-80	S 07°26.691‘/ E 111°36.667‘
Alisol-derived paddy Escalic Hydragrick Anthrosol (Dystric)	China, Yingtan	Silt-clay loam	1300-1900 / sub-tropical (18°C)	*Oryza sativa* (rice), *Trifolium spec.* (clover)	Unknown	Puddling with hand tractor	Unknown	Unknown	40-45	N 28°14.020´/ E 116°53.866´
Cutanic Hyperalic **Alisol** (Chromic)				*Arachis hypogaea* (peanuts), *Sesamum indicum* (sesame), *Ipomoea batatas* (sweet potatoes)	Unknown, liming obverved	Hand tractor	Unknown	Unknown	50-57	N 28°14.035´/ E 116°53.784´
Fluvisol-derived paddy Haplic Gleysol (*Eutric, Arenic,* Densic)	Italy, Zeme	Sandy loam	700-1000 / temperate (13 °C)	*Oryza sativa* (rice), fallow	100 kg ha^{-1} horn and hoofs, 420 kg ha^{-1} NPK*, 75 kg ha^{-1} urea	No puddling, low plouging with tractor	Unknown	Viper, Clincher, Beam, Amistar	79-80	N 45°11.536´/ E 8°40.078´
Endogleyic **Fluvisol** (*Eutric Arenic)*				*Zea mays* (maize), fallow	100kg ha^{-1} horn and hoofs, 500 kg ha^{-1} NPK*, 600kg ha^{-1} urea	Tractor	Unknown	Lumax	79-80	N 45°11.555´/ E 8°40.104´

VIII.

APPENDIX C

Supporting information to Chapter IV

Tab.VIII 1: Soil and sampling site descriptions for Indonesia, China and Italy, including management-specific information, such as on crop rotation and burning.

Country, City	Manage-ment	Soil	Parent material	annual precipitation [mm] / climate	Crop rotation		Burning & site information	Elevation [m a.s.l.]	Coordinates
Indonesia, Sukabumi[a]	paddy	Hydragric Anthrosol (Andic, Dystric, Escalic, Loamic)	andesitic pyroclastics (lahar of Gede volcano)	2300-6700 / tropical (21°C)	*Oryza sativa* (rice), *Oryza sativa* , *Brassica rapa chinensis* (pak choi)	main site	Residual plant material is burnt and spread over the field	870	S 06°52.802‘ / E 106°56.457
						sub site 1	next terrace above main	871	S 06°52.800‘ / E 106°56.463‘
						sub site 2	two terraces above main	871	S 06°52.796‘ / E 106°56.465‘
	non-paddy	Dystric Silandic Andosol (Loamic, Thixotropic)	andesitic pyroclastics (lahar of Gede volcano)	2300-6700 / tropical (21°C)	*Manihot esculenta* (cassava), *Zea maya* (maize)	main site	no burning	981	S 06°52.029‘ / E 106°56.725‘
						sub site 1	10 m from main	972	S 06°52.034‘ / E 106°56.725‘
						sub site 2	30 m from main	969	S 06°52.046‘ / E 106°56.714‘
Indonesia, Jasinga[a]	paddy	Hydragric Anthrosol (Alic, Clayic, Dystric, Escalic)	andesitic tuffs	1900-4700 / tropical (26°C)	*Oryza sativa* (rice), *Oryza sativa* , *Zea maya* (maize)	main site	Residual plant material is burnt and spread over the field	248	S 06°32.205‘ / E 106°31.062‘
						sub site 1	One terrace below main site	240	S 06°32.209‘ / E 106°31.062‘
						sub site 2	Two terraces below main site	238	S 06°32.218‘ / E 106°31.059‘
	non-paddy	Chromic Abruptic Alisol (Pantoclayic, Humic, Hyperalic)	andesitic tuffs	1900-4700 / tropical (26°C)	agroforest: *Manihot esculenta* (cassava), *Musa sp.* (banana), *Leucaena leucocephala* (mimosa)	main site	burning, nearby village (waste?)	238	S 06°32.158‘ / E 106°31.030‘
						sub site 1	110 m from main	236	S 06°32.212‘ / E 106°31.033‘
						sub site 2	140 m from main	238	S 06°32.239‘ / E 106°31.027‘
Indonesia, Ngawi[a]	paddy	Hydragric Anthrosol (Protocalcic, Pantoclayic, Hypereutric, Vertic)	alluvial-volcanic material	2500-6700 / tropical (22-31°C)	*Oryza sativa* (rice), *Oryza sativa* , *Nicotiana sp.* (tobacco)	main site	Rice straw burnt and spread over the field	79	S 07°26.878‘ / E 111°36.599‘
						sub site 1	200 m from main	78	S 07°26.819 / E 111°36.515‘
						sub site 2	500 m from main	77	S 07°27.095‘ / E 111°36.576‘
	non-paddy	Pellic Vertisol (Protocalcic, Grumic, Hypereutric, Humic, Mollic)	alluvial-volcanic material	2500-6700 / tropical (22-31°C)	*Saccharum officinarum* (sugar cane)	main site	Residual plant material is burnt and spread over the field	80	S 07°26.691‘ / E 111°36.667‘
						sub site 1	30 m from main	78	S 07°26.719‘ / E 111°36.672‘
						sub site 2	80 m from main	80	S 07°26.640‘ / E 111°36.530‘

Country, City	Manage-ment	Soil	Parent material	annual precipitation [mm] / climate	Crop rotation		Burning & site information	Elevation [m a.s.l.]	Coordinates
China, Yingtan[a]	paddy	Hydragric Anthrosol (Alic, Endoclayic, Dystric, Escalic, Amphiloamic)	red Cretaceous sandstones with some basalt	1625 / sub-tropical (18°C)	*Oryza sativa* (rice), *Oryza sativa* , *Trifolium sp* . (clover)	main site	burning	45	N 28°14.020´ / E 116°53.866´
						sub site 1	30 m from main	40	N 28°14.034´/ E 116°53.865´
						sub site 2	100 m from main	45	N 28°14.048´/ E 116°53.860´
	non-paddy	Chromic Alisol (Aric, Cutanic, Hyperalic, Pantoloamic)	red Cretaceous sandstones with some basalt	1625 / sub-tropical (18°C)	*Arachis hypogaea* (peanuts), *Sesamum indicum* (sesame), *Ipomoea batatas* (sweet potatoes)	main site	burning, pollution	nd	N 28°14.035´ / E 116°53.784´
						sub site 1	ca. 30 m down hill	57	N 28°14.035´ / E 116°53.784´
						sub site 2	ca. 60 m down hill	50	N 28°14.029´/ E 116°53.830´
China, Cixi[b]	paddy	Endogleyic Hydragric Anthrosol (Hyposodic, Siltic, Thaptomollic)	marine sediments	1325 / subtropical (16°C)	Oryza sativa (rice), *Triticum sp*. (wheat), oilseed rape, barley, broad bean, vegetables	main site	Residual plant material is burnt in piles and spread over the field	6	N 30°10.408´ / E 121°09.180´
						sub site 1	> 50 m from main	6	N 30°10.416´ / E 121°09.169´
						sub site 2	> 50 m from main	6	N 30°10.426´ / E 121°09.153´
	non-paddy	Haplic Cambisol (Eutric, Siltic)	marine sediments	1325 / subtropical (16°C)	*Triticum sp*. (wheat), oilseed rape, barley, broad bean, vegetables	main site	Residual plant material is burnt	5	N 30°10.967´ / E 121°08.706´
						sub site 1	> 50 m from main	5	N 30°10.969´ / E 121°08.724´
						sub site 2	> 50 m from main	5	N 30°10.989´ / E 121°08.724´
Italy, Zeme	paddy	Haplic Gleysol (Eutric, Arenic, Densic)	alluvial sediment	700-1000 / temperate (13 °C)	*Oryza sativa* (rice), fallow	main site	no burning	79	N 45°11.536´ / E 08°40.078´
	non-paddy	Endogleyic Fluvisol (Eutric, Arenic)	alluvial sediment	700-1000 / temperate (13 °C)	*Zea mays* (maize), fallow	main site	no burning, high groundwater level	80	N 45°11.555´ / E 08°40.104´

[a] soil, parent material and climate from Winkler et al. (2016)

[b] soil, parent material and climate from Kölbl et al. (2014), information on burning from Lehndorff et al. (2014)

Tab.VIII 2: Concentration of Black Carbon derived C (BC-C) and single BPCA proportions on total BC in a) paddy soils and b) non-paddy soils. (B3CAs = S tricarboxylic acids, B4CAs = S tetracarboxylic acids, B5CA = pentacarboxylic acid, B6CA = mellitic acid; bdl=below detection limit).

a) site	depth [cm]	horizon	bulk density [g cm^{-3}]	SOC [g kg-1]	BC-C [g C kg-1]	BC-C [g C kg-1 SOC]	B3CAs	B4CAs	B5CA	B6CA
							%			
Andosol paddy	0-9 ± 1	Alp1	0.67 ± 0.07	42.01 ± 2.34	2.39 ± 0.25	56.91 ± 4.48	4.05 ± 0.23	22.93 ± 0.54	40.56 ± 0.89	32.46 ± 0.34
	8-22 ± 1	Alp2	0.76 ± 0.05	42.28 ± 2.53	2.25 ± 0.10	53.26 ± 1.22	4.08 ± 0.11	22.82 ± 0.28	40.42 ± 0.48	32.67 ± 0.50
	22-31 ± 3	Aldp	0.75 ± 0.02	34.77 ± 7.05	1.97 ± 0.11	57.95 ± 9.68	4.75 ± 0.32	24.48 ± 1.09	37.88 ± 0.45	32.89 ± 0.98
	29-35	Bl	0.93 ± 0.08	24.64 ±	1.36	55.28	4.78	28.06	36.54	30.61
	35-50	Bgc1	0.82 ± 0.08	16.38 ±	0.99	60.68	3.92	25.90	40.47	29.71
	50-75	Bgc2	0.77 ± 0.06	9.97 ±	0.35 ± 0.11	46.87 ± 5.92	4.76 ± 3.51	46.59 ± 3.84	23.69 ± 2.34	24.96 ± 2.01
	75-105	Bw	0.72 ± 0.04	6.20 ±	0.18	29.63	2.77	44.26	23.61	29.36
Alisol (I) paddy	0-7 ± 0	Alp1	0.88 ± 0.05	20.04 ± 1.29	1.91 ± 0.28	95.48 ± 13.57	5.43 ± 1.78	26.63 ± 2.84	37.80 ± 5.52	30.14 ± 0.90
	7-17 ± 0	Alp2	0.97 ± 0.03	18.20 ± 0.83	1.66 ± 0.22	91.51 ± 15.68	6.85 ± 1.78	28.69 ± 1.12	34.52 ± 3.80	29.93 ± 1.73
	17-23 ± 3	Alcdp	1.13 ± 0.08	12.95 ± 0.77	1.42 ± 0.11	109.94 ± 2.52	4.75 ± 0.23	27.25 ± 0.25	38.55 ± 0.33	29.45 ± 0.28
	25-48	B(t)gc1	0.97 ± 0.01	10.96 ±	0.89	81.48	4.85	24.24	37.15	33.76
	48-75	B(t)gc2	0.93 ± 0.01	9.90 ±	0.80 ± 0.21	92.90 ± 10.25	5.91 ± 0.51	33.74 ± 3.35	32.95 ± 1.77	27.40 ± 2.88
	75-95+	B(t)gc3	0.88 ± 0.03	6.98 ±	0.43	61.34	3.37	27.40	32.39	36.84
Vertisol paddy	0-8 ± 1	Alp1	0.92 ± 0.92	14.93 ± 1.26	2.08 ± 0.14	140.73 ± 20.70	5.71 ± 0.20	28.32 ± 0.54	35.12 ± 0.58	30.86 ± 0.13
	8-18 ± 2	Alp2	1.04 ± 1.04	11.99 ± 2.09	2.07 ± 0.07	176.58 ± 33.93	5.55 ± 0.28	27.96 ± 0.72	35.29 ± 0.78	31.21 ± 0.39
	18-26 ± 1	Aidp	1.03 ± 1.03	8.25 ± 2.00	2.04 ± 0.26	252.00 ± 28.98	3.81 ± 0.21	25.94 ± 0.16	39.98 ± 0.70	30.28 ± 0.70
	27-47	Bwi	1.00 ± 1.00	5.16 ±	1.65	319.00	4.27	26.94	39.88	28.92
	47-90	Bigc	1.01 ± 1.01	4.76 ±	1.76 ± 0.18	322.24 ± 23.64	4.77 ± 0.09	27.03 ± 0.45	38.50 ± 0.28	29.71 ± 0.33
	90-115+	Bikgc	0.99 ± 0.99	4.64 ±	1.38	296.70	4.55	26.54	40.38	28.53
Cambisol paddy	0-10	Alp1	1.06 ± 0.02	23.20 ± 1.90	1.98 ± 0.25	84.77 ± 14.34	6.75 ± 2.33	29.11 ± 3.84	29.57 ± 2.52	34.57 ± 2.43
	10-16	Alp2	1.20 ± 0.02	18.00 ± 1.80	1.87 ± 0.37	99.48 ± 15.09	12.20 ± 12.89	28.97 ± 5.53	27.72 ± 5.95	31.11 ± 5.92
	16-22	Ardp	1.46 ± 0.03	8.60 ± 0.50	1.15 ± 0.15	133.69 ± 18.57	7.28 ± 3.97	28.82 ± 3.82	30.99 ± 2.93	32.91 ± 4.80
	22-45	Bg	1.49 ± 0.01	4.10 ± 0.60	0.96 ± 0.24	225.19 ± 62.96	10.42 ± 3.83	31.62 ± 3.62	31.07 ± 2.96	26.89 ± 3.21
	45-69	*2Ahgb*	1.53 ± 0.04	5.30 ± 0.60	1.62 ± 0.58	369.87 ± 169.58	6.44 ± 4.39	28.88 ± 3.22	32.90 ± 3.27	31.78 ± 3.71
	69-106	2Blg1	1.45 ± 0.01	3.30 ± 0.40	1.25 ± 0.60	321.10 ± 108.50	9.40 ± 4.38	29.15 ± 3.96	30.79 ± 2.22	30.66 ± 4.88
	106-116	*3Ahlgb*	1.50 ± 0.05	6.00 ± 0.90	3.05 ± 0.09	433.80 ± 13.39	5.88 ± 1.65	31.23 ± 5.12	32.44 ± 5.02	30.45 ± 1.92
Alisol (C) paddy	0-9 ± 1	Alp1	0.81 ± nd	26.2 ± 0.22	1.62 ± 0.13	67.99 ± 1.85	3.09 ± 0.13	24.74 ± 0.13	33.40 ± 0.68	38.77 ± 1.07
	15-20	Ardp	1.59 ± nd	9.4 ±	0.85	89.97	4.09	22.89	35.14	37.89
	20-28	Bl	1.39 ± nd	5.8 ±	0.53	92.37	3.59	27.25	37.39	31.76
	28-42	Bg1	1.42 ± nd	3.5 ±	0.38	108.52	4.24	34.83	33.14	27.79
	60-95+	Bg3	1.21 ± nd	3.1 ±	0.22	71.91	2.83	49.02	25.20	22.95
Gleysol paddy	0-13	Arp1	1.42 ± nd	11.67	0.52	44.77	4.13	34.15	32.87	28.86
	13-25	Arp2	1.49 ± nd	11.75	0.53	44.91	3.58	31.95	35.40	29.07
	25-34	Ardp	1.82 ± nd	6.20	0.41	66.96	3.43	37.31	31.00	28.25
	34-43	Brd	1.93 ± nd	3.23	0.23	71.04	3.84	64.67	31.49	bdl
	43-53	Bg1	1.74 ± nd	2.39	0.18	75.85	1.51	67.77	30.72	bdl
	53-80	Bg2	1.64 ± nd	2.44	0.16	64.58	0.52	58.11	41.36	bdl
	80-90	2CBw	1.61 ± nd	2.16	0.08	37.97	bdl	34.94	65.06	bdl
	90-110+	3C	1.46 ± nd	1.82	0.08	41.34	bdl	bdl	100.00	bdl

b) site	depth [cm]	horizon	bulk density [g cm^{-3}]	SOC [g C kg-1]	BC-C [g C kg-1]	BC-C [g C kg-1 SOC]	B3CAs	B4CAs	B5CA	B6CA
							%			
Andosol non-paddy	0-10 ± 3	Ah1	0.67 ± 0.05	37.27 ± 3.13	0.98 ± 0.09	26.24 ± 2.57	3.57 ± 0.29	22.28 ± 0.97	39.55 ± 1.57	34.60 ± 0.66
	7-20 ± 3	Ah2	0.65 ± 0.04	34.59 ± 1.48	1.03 ± 0.15	29.56 ± 3.36	3.55 ± 0.40	22.13 ± 0.90	39.36 ± 1.80	34.96 ± 0.99
	16-28 ± 8	BwAh	0.51 ± 0.05	17.43 ±	0.38 ± 0.38	21.56 ± 7.56	2.94 ± 0.08	35.82 ± 2.99	28.85 ± 2.50	32.40 ± 1.08
	28-56	Bw1	0.53 ± 0.06	13.54 ±	0.26	19.16	2.00	33.08	29.89	35.03
	56-78	Bw2	0.63 ± 0.11	13.77 ±	0.30 ± 0.30	32.55 ± 10.29	2.21 ± 0.06	46.72 ± 0.98	25.47 ± 0.48	25.59 ± 1.32
	78-100+	Bw3	0.63 ± 0.17	8.80 ±	0.29	15.45	3.72	23.04	39.08	34.16
Alisol (I) non-paddy	0-9 ± 2	Ah1	0.83 ± 0.17	23.86 ± 3.35	1.89 ± 0.11	80.73 ± 15.78	5.14 ± 0.48	26.72 ± 0.39	35.67 ± 0.86	32.48 ± 1.56
	7-16 ± 2	Ah2	0.88 ± 0.15	21.08 ± 2.97	1.94 ± 0.24	93.74 ± 22.35	4.82 ± 0.27	26.61 ± 0.35	35.68 ± 0.55	32.89 ± 0.94
	14-22	B(t)o1	0.94 ± 0.16	17.20 ±	1.61 ± 0.10	92.56 ± 2.86	5.00 ± 0.43	26.72 ± 0.41	36.41 ± 0.40	31.87 ± 0.46
	22-46	B(t)o2	0.89 ± 0.08	9.43 ±	0.70	73.87	4.52	27.51	35.32	32.64
	46-63	B(t)o3	0.92 ± 0.03	7.79 ±	0.57 ± 0.17	61.53 ± 11.90	4.09 ± 0.31	31.49 ± 0.55	32.80 ± 0.55	31.62 ± 0.58
	63-83	B(t)o4	0.87 ± 0.06	6.78 ±	0.36	53.76	2.32	40.56	29.46	27.66
	83-100+	B(t)o5	1.11 ± 0.00	4.24 ±	0.32	76.32	1.60	31.88	24.21	42.31
Vertisol non-paddy	0-10 ± 0	Ah1	0.82 ± 0.11	15.11 ± 1.49	2.84 ± 0.43	187.57 ± 9.93	4.81 ± 0.64	26.39 ± 0.75	37.92 ± 3.00	30.88 ± 1.60
	10-21 ± 2	Ah2	0.94 ± 0.15	13.63 ± 0.53	2.37 ± 0.07	173.65 ± 4.50	5.69 ± 0.42	27.43 ± 1.24	34.59 ± 1.31	32.29 ± 0.48
	23-29	Bw	0.96 ± 0.05	8.98 ±	2.55 ± 0.36	229.38 ± 20.74	4.21 ± 0.18	25.44 ± 1.06	39.94 ± 1.03	30.41 ± 2.18
	29-55	Bwi1	0.97 ± 0.05	7.14 ±	1.88	262.82	4.39	26.28	39.90	29.43
	55-100	Bwi2	1.02 ± 0.03	5.76 ±	1.57 ± 0.22	275.11 ± 37.07	4.70 ± 0.11	26.99 ± 0.22	38.46 ± 0.12	29.86 ± 0.22
	100-120+	Bwi3	0.99 ± 0.06	4.78 ±	1.67	349.73	4.58	30.57	39.53	25.32
Cambisol non-paddy	0-12	Ap1	1.25 ± 0.06	11.00 ± 1.90	1.08 ± 0.33	99.95 ± 22.17	5.09 ± 0.75	28.26 ± 3.46	35.20 ± 4.97	31.44 ± 4.97
	12-17	Ap2	1.32 ± 0.03	7.10 ± 1.80	0.84 ± 0.17	119.83 ± 34.44	5.48 ± 1.11	33.64 ± 6.73	32.59 ± 4.98	28.29 ± 3.59
	17-23	Bw1	1.38 ± 0.01	5.30 ± 0.80	0.52 ± 0.14	113.11 ± 31.11	5.00 ± 1.18	36.34 ± 4.63	30.98 ± 2.68	27.68 ± 4.09
	23-45	Bw2	1.39 ± 0.03	5.30 ± 0.40	0.43 ± 0.21	80.59 ± 28.27	5.01 ± 1.09	41.07 ± 3.67	28.04 ± 3.26	25.88 ± 2.59
	45-70	Bwl1	1.42 ± 0.02	4.80 ± 0.10	0.37 ± 0.08	76.57 ± 15.85	4.45 ± 1.05	43.55 ± 4.98	27.30 ± 2.56	24.70 ± 3.63
	70-100	Bwl2	1.42 ± 0.01	4.20 ± 0.50	0.43 ± 0.14	91.24 ± 30.36	3.84 ± 1.01	44.41 ± 3.55	25.27 ± 3.52	26.48 ± 1.67
Alisol (C) non-paddy	0-10 ± 3	Alp1	1.13	7.13 ± 0.06	0.78	109.22	3.35	30.58	30.33	35.73
	20-28	Bl	1.34	2.8 ±	0.23	83.68	2.45	29.25	37.11	31.20
	28-42	Bg1	1.23	1.5 ±	0.11	78.18	1.28	69.66	29.05	bdl
	60-95+	Bg3	1.37	1.4 ±	0.12	86.56	2.41	40.93	41.16	15.50
Fluvisol non-paddy	0-15	Ap1	1.48	6.87	0.38	55.63	2.50	40.29	28.00	29.21
	15-30	Ap2	1.66	7.56	0.40	52.94	3.56	36.51	30.13	29.81
	30-70	Bgw	1.55	2.26	0.07	31.56	bdl	bdl	100.00	bdl
	70-85	2Bgw	1.51	1.51	0.10	68.93	bdl	bdl	100.00	bdl
	85-110+	3C	1.49	0.46	0.04	85.89	bdl	bdl	100.00	bdl

Tab.VIII 3: N forms in rice, rice char and paddy and non-paddy topsoils, main sites only (na: not analyzed, nd: not detectable).

site, sample info	management	depth [cm]	horizon	N (EA) [g kg-1]	N 1s (XPS) [g kg-1]	aromatic N ~399 eV [%]	amid N ~400 eV [%]	primary N ~402 eV [%]
rice straw				3.8	10.20 ± 1.20	5.3 ± 2.7	86.7 ± 7.2	2.9 ± 6.4
rice char					5.50 ± 1.45	56.7 ± 24.1	40.2 ± 22.2	3.1 ± 4.3
Andosol	paddy	0-8	Alp1	4.17	10.63 ± 2.43	5.60 ± 2.50	82.60 ± 13.00	11.80 ± 12.50
Alisol (I)	paddy	0-7	Alp1	1.88	3.23 ± 1.25	nd	63.90	36.10
Vertisol	paddy	0-8	Alp1	1.48		na		
Cambisol	paddy					na		
Alisol (C)	paddy	0-8	Alp1	2.62	13.73 ± 1.86	2.90 ± 4.00	80.80 ± 7.50	16.40 ± 9.50
Gleysol	paddy	0-13	Arp1	1.09	7.87 ± 0.45	7.90 ± 7.70	74.60 ± 13.40	17.50 ± 5.70
Andosol	non-paddy	0-7	Ah1	3.55	13.47 ± 2.84	0.90 ± 1.20	91.10 ± 3.20	8.00 ± 4.40
Alisol (I)	non-paddy	0-7	Ah1	1.85	2.57 ± 0.96	0.00 ± 0.00	79.70 ± 2.40	20.30 ± 2.40
Vertisol	non-paddy	0-10	Ah1	1.15	2.33 ± 2.25	nd	nd	nd
Cambisol	non-paddy					na		
Alisol (C)	non-paddy	0-12	Alp1	0.65	3.73 ± 2.01	nd	nd	nd
Fluvisol	non-paddy	0-15	Ap1	0.78	4.87 ± 1.40	6.60 ± 3.30	72.20 ± 7.10	21.20 ± 6.40

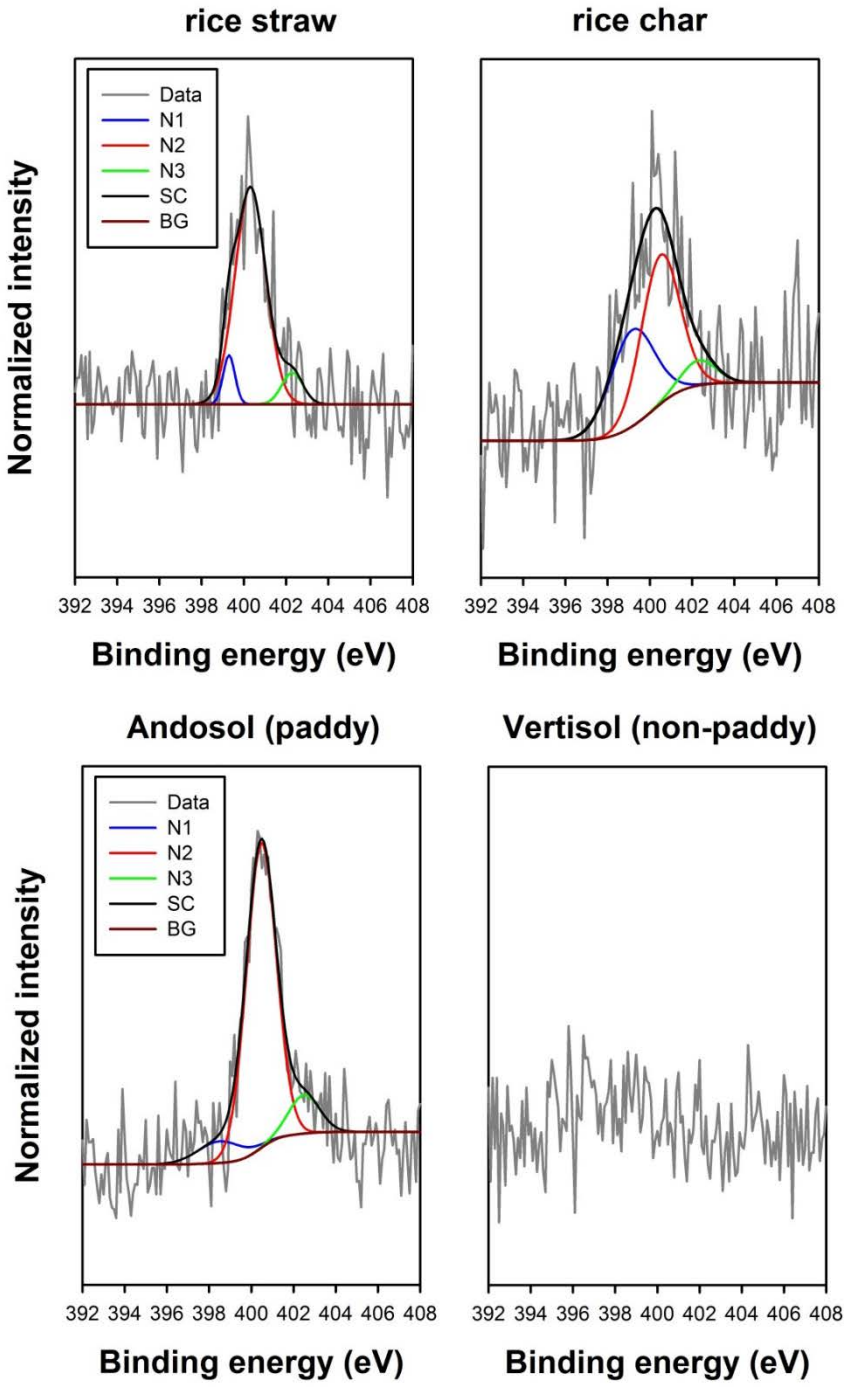

Fig.VIII 1: XPS spectra, including fittings for aromatic N (N1), amide N (N2), and primary N (N3) exemplarily for rice straw, rice char, the Andosol under paddy management and the Vertisol under non-paddy management

IX.

APPENDIX D

Supporting information to Chapter V

Supporting information to the Greenhouse experiment.

1. Introduction of the Greenhouse experiment

Source references

The greenhouse experiment was part of my PhD thesis. The analytical part was part of the master thesis of Nadine Kubsch “Nitrogen sequestration in microbial biomass residues: Short-term fertilizer N incorporation into amino sugars in rice paddy soils”. I was supervisor of this master thesis. Therefore, I partly copied and partly modified text passages from the master thesis of Nadine Kubsch in Appendix D of this thesis.

2. Materials und methods of the Greenhouse experiment

Experiment set-up and sampling

The greenhouse experiment (Fig.IX 1) was carried out with soil samples from the site in Zeme (Italy) described in chapters above. The experimental setup (Fig.IX 2)

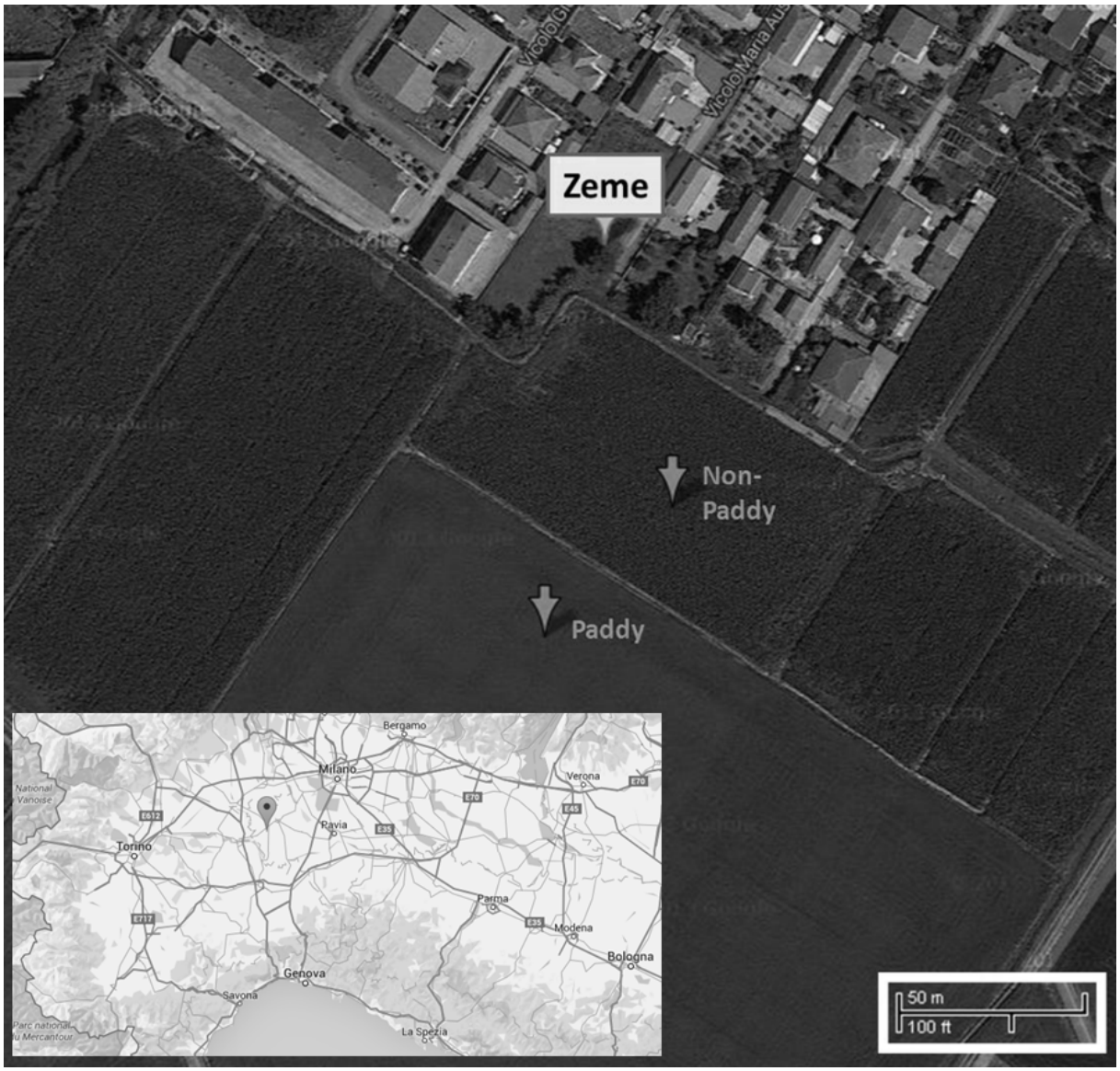

Fig.IX 1: The picture shows the location of the study site (Zeme, Italy) from which the soil samples were taken and demonstrates that the paddy and non-paddy soil are located directly next to each other. The small picture within the large one shows where Zeme is located. Source: Master thesis Nadine Kubsch

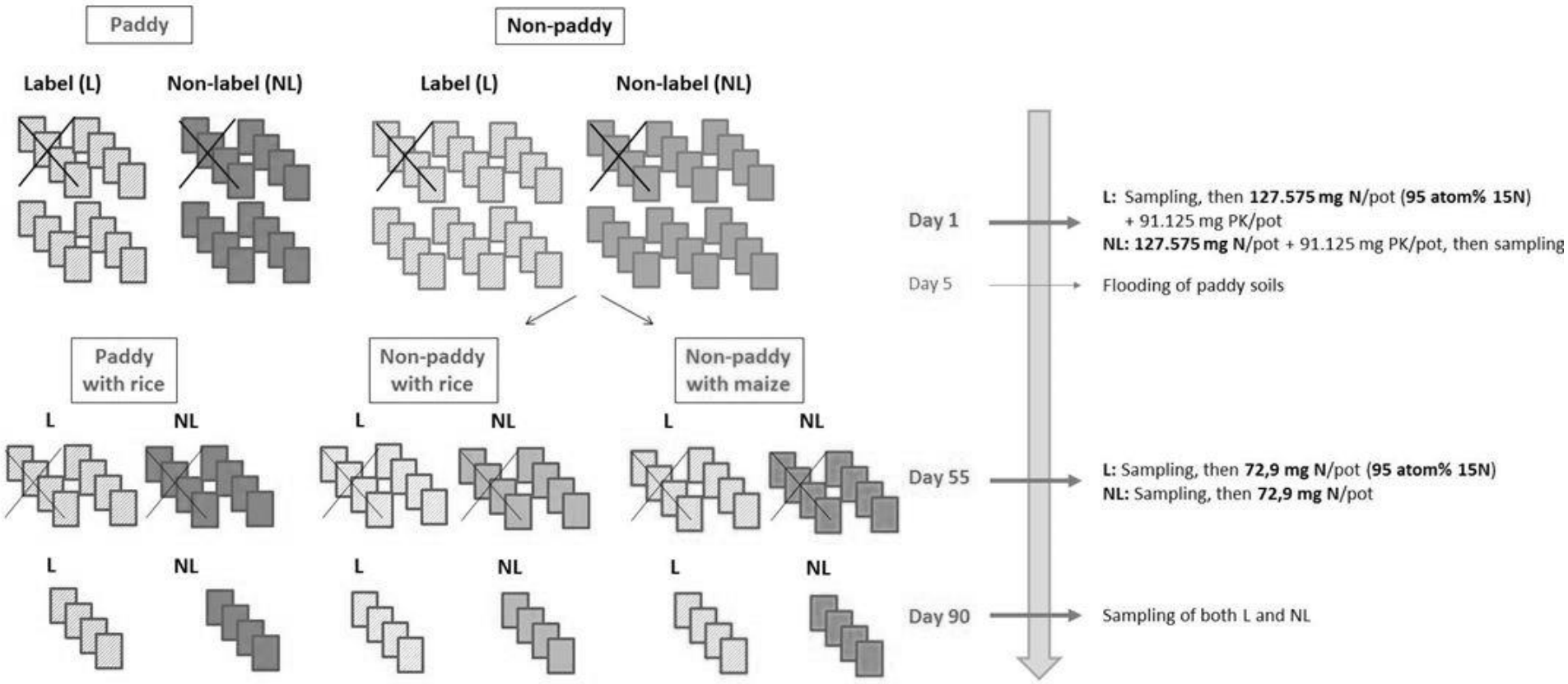

Fig.IX 2: Schematic diagram of the setup of the greenhouse experiment next to a time scale explaining at which points of time the management measures sampling, fertilization and flooding were implemented; pots that are crossed represent taken samples

Analysis

All samples were analyzed for total N and C content and for the concentration and ^{15}N enrichment of amino sugars glucosamine, galactosamine, mannosamine and muramic acid were determined. On this data basis, it was possible to calculate the ^{15}N recovery in bulk soil and the incorporation of applied ^{15}N-labeled fertilizer into amino sugars.

C, N and $\delta^{15}N$ analyses

The analysis of total C and N was carried out in duplicate with an elemental analyzer (Vario MICRO cube, Elementar Analysensysteme GmbH, Hanau, Germany). We determined the $\delta^{15}N$ isotope ratio in duplicate by elemental analysis - isotope ratio mass spectrometry (EA-IRMS; Flash EA 1112 coupled to DeltaV Advantage, ThermoFisher GmbH, Schwerte, Germany). The isotope ratio was expressed as $\delta^{15}N$ in ‰ using $\delta^{15}N$ of atmospheric air as a reference. Analytical drift was corrected by external standards acetanilide (1.18 ‰ $\delta^{15}N$) and ammonium sulfate (20.3 ‰ $\delta^{15}N$).

Analyses of amino sugars

We analyzed four amino sugars: glucosamine (Glu), galactosamine (Gal), mannosamine (Man) and muramic acid (MurA). Amino sugars were detected according to Amelung and Zhang (1996). Samples were hydrolyzed with 6 M hydrochloric acid and recovery standard (myo-inositol) was added. The hydrolysate was filtered and dried. The purification was conducted in two steps, first solution was adjusted to pH 6.6-6.8 and centrifuged, and then the freeze-dried supernatant was washed with methanol. Samples were derivatized to aldononitrile derivates using hydroxylamine hydrochloride and dimethylaminopyridine, followed by acetic anhydride addition. After derivatization the quantification standard was added (β-endosulfan).

The four amino sugar derivatives were measured with a gas chromatograph - flame ionization detector (GC – FID) (Agilent 6890, Agilent GmbH, Böblingen, Germany) using a capillary column (Optima-5 MS; 30 m, I.D. 0.25 mm; Macherey Nagel). Detailed method description and temperature program see Zhang und Amelung (1996). The recovery of the first internal standard myo-inositol exceeded 70 % in all runs.

Calculations

For determination of the recovery of applied ^{15}N fertilizer (urea) in bulk soil, firstly the relative abundance of ^{15}N to ^{14}N was calculated as

$$^{15}N\ abundance_{soil} = {}^{15}N/ ({}^{14}N+{}^{15}N) = ((\delta^{15}N/1000+1)*R_{Std})/ ((\delta^{15}N/1000+1)*R_{Std}) +1) \quad (5)$$

with R_{Std} as standard of 0.0036765 for correction of natural abundance of ^{15}N in the atmosphere (*Epron* et al., 2011; *Providoli* et al., 2005). Secondly, excess $^{15}N_{soil}$ was calculated to relate $^{15}N\ abundance_{soil}$ to the total N content of the bulk soil and to the natural ^{15}N abundance ($^{15}N\ abundance_{control}$), which was originally in soil. $^{15}N\ abundance_{control}$ was the mean of the 4 replicates of the non-labeled treatments from each management set (paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize). Therewith, following equation was obtained (*Epron* et al., 2011):

$$Excess\ ^{15}N_{soil} = (^{15}N\ abundance_{label} - {}^{15}N\ abundance_{control}) * N_{tot}\ [g\ kg^{-1}] \quad (6)$$

The recovery of applied ^{15}N fertilizer in bulk soil (expressed as percentage) was then calculated as following (*Providoli* et al., 2005):

$$^{15}N\ recovery_{soil} = excess\ ^{15}N_{soil}\ [g\ kg^{-1}] * soil\ quantity\ [kg] * 100/\ ^{15}N_{applied}\ [g] \quad (7)$$

This equation includes soil quantity as amount of soil in the pots and $^{15}N_{applied}$ as amount of applied N fertilizer multiplied with ^{15}N atom-%.

The atom percentage excess (APE) is the proportion of ^{15}N isotope enrichment versus a control. To assess the relative abundance of ^{15}N in the dominant molecule fragment, the mass of the respective fragment *F* and the mass of the corresponding *F* + *n* ion with *n* for the number of N atoms were determined by GC/MS. Amino sugar molecules only contain one N atom, therefore n is equal to 1. The abundance ratio R of *F*+1 to *F* was determined for each compound. This abundance ratio and the one of the controls were used to calculate the APE as following (*He* et al., 2006):

$$APE = (R_{labeled} - R_{control})*100/ (1+ (R_{labeled} - R_{control})) \quad (8)$$

APE includes the R of ^{15}N labeled samples as $R_{labeled}$ and R of controls as $R_{control}$. The APE was determined for glucosamine, galactosamine and muramic acid.

Incorporation of ^{15}N fertilizer into amino sugars is expressed by ^{15}N recovery in amino sugar (^{15}N recovery$_{AS}$). Therefore, we modified the calculation process of ^{15}N recovery soil (formulas 5 - 7) as following:

$$^{15}N \text{ abundance}_{AS} = (F+1)/ (F + (F+1)) \quad (9)$$

With ^{15}N abundance$_{AS}$, excess ^{15}N in amino sugar is calculated as following:

$$\text{Excess } ^{15}N_{AS} = (^{15}N \text{ abundance}_{AS(label)} - ^{15}N \text{ abundance}_{AS(control)}) * \text{amino sugar N [g kg}^{-1}] \quad (10)$$

Amino sugar-N is included as the concentration of amino sugar in soil multiplied with its mass fraction of N. In muramic acid this mass fraction accounts for 0.05018 and in the other amino sugars for 0.0848. ^{15}N recovery$_{AS}$ can then be calculated with formula (7) when replacing excess $^{15}N_{soil}$ by excess $^{15}N_{AS}$.

Statistic

SigmaPlot 13.0 for Windows (Systat Software) was used for descriptive and inferential statistics as well as for the production of the graphs. To test if the differences between the management systems were significant, one-way analysis of variance (ANOVA) with Bonferroni t-test and Holm-Sidak Method was run if parameters passed Normality-Test (Shapiro-Wilk Test). ANOVA on Ranks was used with Kuskal-Wallis-H Test and Dunnett's Method if the Normality-Test failed. The tests were run at a confidence level of 95 %. The results were considered to be significant at $P < 0.05$ and highly significant at $P < 0.01$. All tables were created with Microsoft Excel 2010.

3. Results of the Greenhouse experiment

The total N content ranged between 1.0 ± 0.0 and 1.1 ± 0.1 g kg^{-1} in the paddy soils and between 0.8 ± 0. and 0.8 ± 0.0 g kg^{-1} in the non-paddy soils. Total N content and C to N ratio were stable over the whole time of the experiment in all treatments (Tab.IX **1**). The total amino sugar content comprised the sum of glucosamine, mannosamine, galactosamine and muramic acid. The ancient paddy soil (P-R) contained 18.3 ± 2.9 to 24.1 ± 6.8 g amino sugar-N kg^{-1} N over the time of the experiment**.**. Therewith, the amino sugar-N content accounted for 1.4-3.2 % of total N in the paddy soil over the whole period of the experiment. The amino sugar-N content in the non-paddy soil cropped with rice (NP-R) ranged between 41.5 ± 6.7 and 50.2 ± 18.4 g N kg^{-1} N and between 30.75 ± 3.5 and 66.8 ± 8.0 g kg^{-1} N in the non-paddy soil cropped with maize (NP-M). The amino sugar-N content accounted for about 4.2-8.6 % of the total N content in the non-paddy soils (NP-R ad NP-M) (Tab.IX **3**). The amino sugar-N content in the ancient paddy soil (P-R) was thus about half as low as the content in both non-paddy soils. This difference between the paddy soil and the non-paddy soils was significant ($P < 0.001$). The difference between both non-paddy soils was not significant ($P = 0.713$).

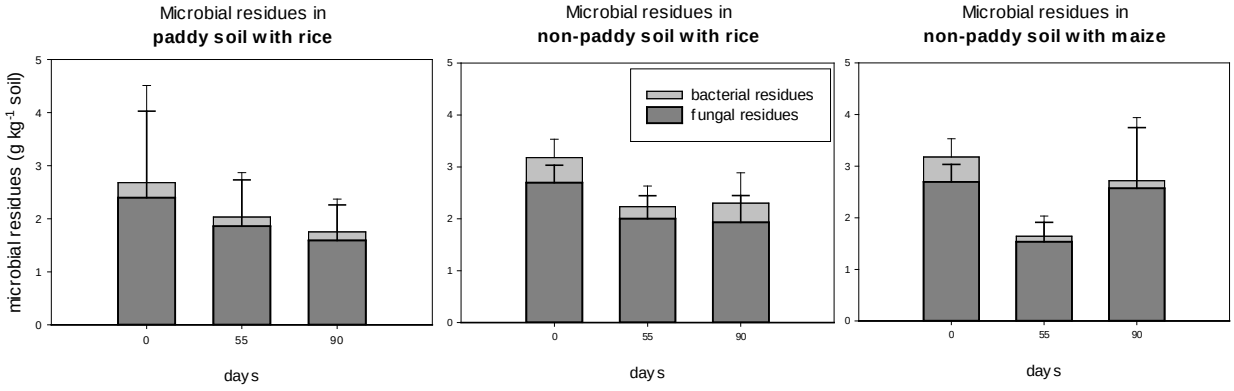

Fig.IX 3: Microbial residue contents in the paddy soil with rice (P-R), non-paddy soil cultivated with rice (NP-R) and non-paddy soil with maize (NP-M) at day 1 (pre-seeding), day 55 (tillering) and day 90 (flowering). The error bars represent standard deviations of the greenhouse pot replicates. (n=4)

^{15}N recovery in bulk soil accounted for 22.4 ± 3.49 % of the applied amount in the ancient paddy soil after the first labeling (day 55) and 22.6 ± 1.5 % after the second labeling (day 90). In the non-paddy soil with rice (NP-R), 26.2 ± 7.5 % of applied ^{15}N were found on day 55 and 22.1 ± 6.4 % were found on day 90. The ^{15}N recovery in the non-paddy soil with maize (NP-M) was 33.5 ± 5.1 % on day 55 and 27.8 ± 5.6 % on day 90 (Fig.IX 4).

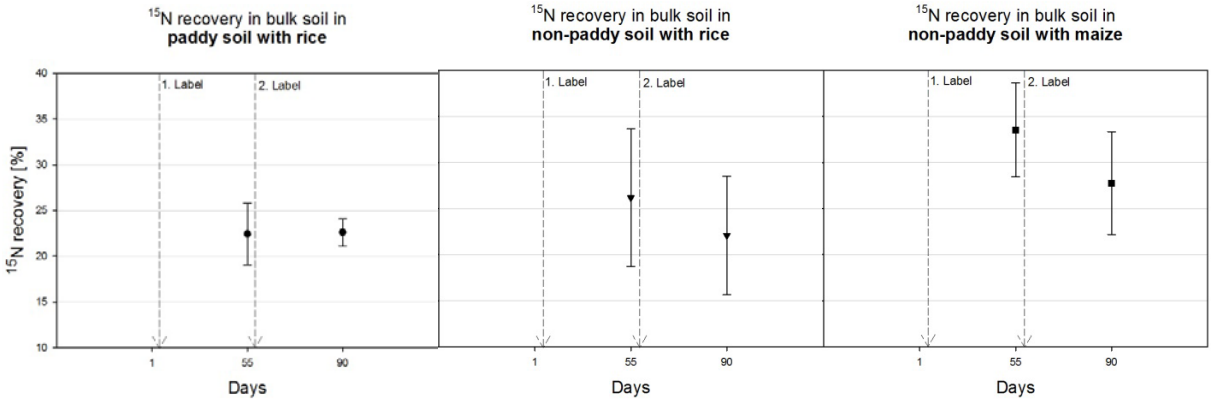

Fig.IX 4: ^{15}N recovery in bulk soil in the paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize at day 1 (pre-seeding; non-labeled), day 55 (tillering; 55 days after first labeling, with 127.5 mg N urea per pot) and day 90 (flowering; 35 days after second labeling, with 72.9 mg N urea per pot). The error bars represent standard deviations of the pot replicates (n=4).

Tab.IX 1: Mean total N content and C/N ± standard deviations (n=4) in the paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize at day 1 (pre-seeding), day 55 (tillering) and day 90 (flowering) (n=4)

Time	**Paddy soil (rice)**	**Non-paddy soil (rice)**	**Non-paddy soil (maize)**
Total N content (g N kg^{-1} soil)			
Day 1	1.09 ± 0.10	0.82 ± 0.04*[1]	0.82 ± 0.04*[1]
Day 55	1.00 ± 0.04	0.81 ± 0.07	0.81 ± 0.07
Day 90	1.11 ± 0.13	0.75 ± 0.03	0.77 ± 0.03
C/N			
Day 1	10.15 ± 0.10	8.43 ± 0.26*[2]	8.43 ± 0.26*[2]
Day 55	10.70 ± 0.16	8.79 ± 0.07	8.94 ± 0.39
Day 90	10.59 ± 0.03	8.78 ± 0.17	8.53 ± 0.18

Asterisks show that both values are the same respectively (at day 1 no differentiation between non-paddy soils, because planting of rice and maize did not occur yet)

Tab.IX 2: Excess ^{15}N and ^{15}N recovery of applied labeled urea in bulk soil ± standard deviation in the paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize at day 1 (only for excess ^{15}N), day 55 (tillering; 55 days after first labeling, with 127.575 mg N urea per pot) and day 90 (flowering; 35 days after second labeling, with 72.9 mg N urea per pot. (n = 4)

Time	**Paddy soil (rice)**	**Non-paddy soil (rice)**	**Non-paddy soil (maize)**
Excess ^{15}N (mg kg^{-1} soil)			
Day 1	0.00 ± 0,01	0.04 ± 0,07*	0.04 ± 0.07*
Day 55	8.28 ± 1,25	9.16 ± 2,61	11.74 ± 1.78
Day 90	13.11 ± 0,86	12.14 ± 3,54	15.27 ± 3.07
^{15}N recovery (%)			
Day 55	22.42 ± 3.39	26.18 ± 7.45	33.53 ± 5.08
Day 90	22.59 ± 1.49	22.08 ± 6.43	27.76 ± 5.58

Asterisks show that both values are the same respectively (at day 1 no differentiation between non-paddy soils, because planting of rice and maize did not occur yet)

Tab.IX 3: Mean amino sugar-N content ± standard deviation in the paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize at day 1 (pre-seeding), day 55 (tillering) and day 90 (flowering). (n = 4)

Amino sugars Time	**Paddy soil (rice)** (g AS-N kg^{-1} N)	**Non-paddy soil (rice)** (g AS-N kg^{-1} N)	**Non-paddy soil (maize)** (g AS-N kg^{-1} N)
Sum			
Day 1	18.28 ± 2.90	49.59 ± 4.52*	49.59 ± 4.52*
Day 55	24.06 ± 6.84	41.47 ± 6.70	30.65 ± 3.48
Day 90	21.64 ± 4.00	50.17 ± 18.40	66.75 ± 7.96
Glucosamine			
Day 1	8.16 ± 1.77	30.91 ± 2.86*[1]	30.91 ± 2.86*[1]
Day 55	11.24 ± 1.54	22.14 ± 4.59	20.60 ± 2.79
Day 90	9.22 ± 1.42	24.03 ± 7.33	45.23 ± 4.40
Galactosamine			
Day 1	9.65 ± 1.62	16.34 ± 1.07*[2]	16.34 ± 1.07*[2]
Day 55	13.11 ± 2.51	15.91 ± 0.52	11.55 ± 1.54
Day 90	10.50 ± 2.03	18.85 ± 5.04	26.36 ± 1.66
Muramic acid			
Day 1	0.10 ± 0.03	0.66 ± 0.03*[3]	0.66 ± 0.03*[3]
Day 55	0.25 ± 0.05	0.48 ± 0.02	0.16 ± 0.01
Day 90	0.21 ± 0.05	0.49 ± 0.08	0.27 ± 0.04
Mannosamine			
Day 1	0.88 ± 0.10	0.61 ± 0.08*[4]	0.61 ± 0.08*[4]
Day 55	1.27 ± 0.22	0.90 ± 0.11	0.53 ± 0.10
Day 90	0.96 ± 0.18	1.06 ± 0.22	1.05 ± 0.07

Asterisks show that respective values are the same (at day 1 was no differentiation between non-paddy soils, because planting of rice and maize did not occur yet)

Tab.IX 4: Means of glucosamine-to-galactosamine (Glu/Gal) and glucosamine-to-muramic acid (Glu/Mur) ratios ± standard deviation in the paddy soil with rice, non-paddy soil with rice and non-paddy soil with maize at day 1 (pre-seeding), day 55 (tillering) and day 90 (flowering). (n = 4)

Time	**Paddy soil (rice)**	**Non-paddy soil (rice)**	**Non-paddy soil (maize)**
Glu/Gal			
Day 1	0.84 ± 0.05	1.90 ± 0.20[*1]	1.90 ± 0.20[*1]
Day 55	0.87 ± 0.07	1.39 ± 0.26	1.78 ± 0.05
Day 90	0.89 ± 0.10	1.27 ± 0.06	1.71 ± 0.09
Glu/Mur			
Day 1	51.39 ± 8.28	27.79 ± 3.53[*2]	27.79 ± 3.53[*2]
Day 55	27.02 ± 3.37	27.62 ± 6.81	74.50 ± 9.71
Day 90	26.13 ± 3.80	28.86 ± 4.52	99.68 ± 6.85

Asterisks show that respective values are the same (at day 1 was no differentiation between non-paddy soils, because planting of rice and maize did not occur yet)

X.

REFERENCES

Abe T, Maie N, Watanabe A (2005) Investigation of humic acid N with X-ray photoelectron spectroscopy: Effect of acid hydrolysis and comparison with 15N cross polarization/magic angle spinning nuclear magnetic resonance spectroscopy. Organic Geochemistry 36(11):1490–1497. doi:10.1016/j.orggeochem.2005.07.005

Ainsworth CC, Sumner ME, Hurst VJ (1985) Effect of aluminum substitution in goethite on phosphorus adsorption: I. Adsorption and isotopic exchange. Soil Science Society of America Journal(49):1142–1149

Allen DE, Kingston G, Rennenberg H, Dalal RC, Schmidt S (2010) Effect of nitrogen fertilizer management and waterlogging on nitrous oxide emission from subtropical sugarcane soils. Agriculture, Ecosystems & Environment 136(3-4):209–217. doi:10.1016/j.agee.2009.11.002

Amelung W, Brodowski S, Sandhage-Hofmann A, Bol R (2008) Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. Advances in agronomy 100:155–250

Amelung W, Miltner A, Zhang X, Zech W (2001) Fate of microbial residues during litter decomposition as affected by minerals. Soil Science 166(9):598–606. doi:10.1097/00010694-200109000-00003

Amelung W, Zhang X (2001) Determination of amino acid enantiomers in soils. Soil Biology and Biochemistry 33(4–5):553–562. doi:10.1016/S0038-0717(00)00195-4

Amelung W, Zhang X, Flach KW (2006) Amino acids in grassland soils: Climatic effects on concentrations and chirality. Geoderma 130(3–4):207–217. doi:10.1016/j.geoderma.2005.01.017

Amelung W (2003) Nitrogen biomarkers and their fate in soil. Z. Pflanzenernähr. Bodenk. 166(6):677–686. doi:10.1002/jpln.200321274

Amelung W, Zhang X, Zech W, Flach KW (1999) Amino Sugars in Native Grassland Soils along a Climosequence in North America. Soil Science Society of America Journal 63(1):86. doi:10.2136/sssaj1999.03615995006300010014x

Appuhn A, Joergensen RG (2006) Microbial colonisation of roots as a function of plant species. Soil Biology and Biochemistry 38(5):1040–1051. doi:10.1016/j.soilbio.2005.09.002

Bada JL (1985) Amino acid racemization dating of fossil bones. Annual Review of Earth and Planetary Sciences 13:241

Batjes NH (2014) Total carbon and nitrogen in the soils of the world. Eur J Soil Science 65(1):10–21. doi:10.1111/ejss.12114_2

Black CA (1965) Front Matter. In: Black CA (ed) Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. American Society of Agronomy, Soil Science Society of America, [S.l.]

Black CA (ed) (1965) Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. Agronomy Monograph. American Society of Agronomy, Soil Science Society of America, [S.l.]

Blagodatskaya EV, Anderson T-H (1998) Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO2 of microbial communities in forest soils. Soil Biology and Biochemistry 30(10-11):1269–1274. doi:10.1016/S0038-0717(98)00050-9

Boddey RM, Oliveira OC de, Urquiaga S, Reis VM, Olivares FL de, Baldani VLD, Döbereiner J (1995) Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. Plant and Soil 174(1-2):195–209

Borggaard OK (1982) The influence of iron oxides on the surface area of soil. Journal of Soil Science 33(3):443–449

Borggaard OK, Jdrgensen SS, Moberg JP, Raben-Lange B (1990) Influence of organic matter on phosphate adsorption by aluminium and iron oxides in sandy soils. Journal of Soil Science 41(3):443–449

Brady NC (ed) (1987) Advances in agronomy. Vol. 41. Advances in Agronomy. Academic Press, San Diego

Bräuer T, Grootes PM, Nadeau M-J, Andersen N (2013) Downward carbon transport in a 2000-year rice paddy soil chronosequence traced by radiocarbon measurements. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 294:584–587

Brodowski S, John B, Flessa H, Amelung W (2006) Aggregate-occluded black carbon in soil. Eur J Soil Science 57(4):539–546. doi:10.1111/j.1365-2389.2006.00807.x

Brodowski S, Rodionov A, Haumaier L, Glaser B, Amelung W (2005) Revised black carbon assessment using benzene polycarboxylic acids. Organic Geochemistry 36(9):1299–1310. doi:10.1016/j.orggeochem.2005.03.011

Brodowski S, Amelung W, Haumaier L, Abetz C, Zech W (2005) Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. Geoderma 128(1-2):116–129. doi:10.1016/j.geoderma.2004.12.019

Brodowski S, Amelung W, Haumaier L, Zech W (2007) Black carbon contribution to stable humus in German arable soils. Geoderma 139(1-2):220–228. doi:10.1016/j.geoderma.2007.02.004

Brodowski S, Amelung W, Lobe I, Du Preez CC (2005) Losses and biogeochemical cycling of soil organic nitrogen with prolonged arable cropping in the South African Highveld—evidence from D-and L-amino acids. Biogeochemistry 71(1):17–42

Bundt M, Widmer F, Pesaro M, Zeyer J, Blaser P (2001) Preferential flow paths: biological ‘hot spots’ in soils. Soil Biology and Biochemistry 33(6):729–738. doi:10.1016/S0038-0717(00)00218-2

Cassman KG, Dobermann A, Cruz PCS, Gines GC, Samson MI, Descalsota JP, Alcantara JM, Dizon MA, Olk DC (1996) Soil organic matter and the indigenous nitrogen supply of intensive irrigated rice systems in the tropics. Plant and Soil 182(2):267–278. doi:10.1007/BF00029058

Cassman KG, Gines GC, Dizon MA, Samson MI, Alcantara JM (1996) Nitrogen-use efficiency in tropical lowland rice systems: contributions from indigenous and applied nitrogen. Field Crops Research 47(1):1–12. doi:10.1016/0378-4290(95)00101-8

Chantigny MH, Angers DA, Prévost D, Vézina L-P, Chalifour F-P (1997) Soil Aggregation and Fungal and Bacterial Biomass under Annual and Perennial Cropping Systems. Soil Science Society of America Journal 61(1):262. doi:10.2136/sssaj1997.03615995006100010037x

Chen C, Dynes JJ, Wang J, Sparks DL (2014) Properties of Fe-organic matter associations via coprecipitation versus adsorption. Environmental Science and Technology(48):13751–13759

Cheng C-H, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black carbon by biotic and abiotic processes. Organic Geochemistry 37(11):1477–1488. doi:10.1016/j.orggeochem.2006.06.022

Cheshire MV, Dumat C, Fraser AR, Hillier S, Staunton S (2000) The interaction between soil organic matter and soil clay minerals by selective removal and controlled addition of organic matter. European Journal of Soil Science 51(3):497–509

Cock JH (1982) Cassava. A basic energy source in the tropics. Science (New York, N.Y.) 218(4574):755–762

Constantinides M, Fownes JH (1994) Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biology and Biochemistry 26(1):49–55. doi:10.1016/0038-0717(94)90194-5

Cucu MA, Said-Pullicino D, Maurino V, Bonifacio E, Romani M, Celi L (2013) Influence of redox conditions and rice straw incorporation on nitrogen availability in fertilized paddy soils. Biology and Fertility of Soils 50(5):755–764. doi:10.1007/s00374-013-0893-4

Cusack DF, Chadwick OA, Hockaday WC, Vitousek PM (2012) Mineralogical controls on soil black carbon preservation. Global Biogeochem. Cycles 26(2):n/a-n/a. doi:10.1029/2011GB004109

Czimczik CI, Preston CM, Schmidt MWI, Werner RA, Schulze E-D (2002) Effects of charring on mass, organic carbon, and stable carbon isotope composition of wood. Organic Geochemistry 33(11):1207–1223. doi:10.1016/S0146-6380(02)00137-7

Dick RP (1992) A review. Long-term effects of agricultural systems on soil biochemical and microbial parameters. Agriculture, Ecosystems & Environment 40(1-4):25–36. doi:10.1016/0167-8809(92)90081-L

Dijkstra P, Ishizu A, Doucett R, Hart SC, Schwartz E, Menyailo OV, Hungate BA (2006) 13C and 15N natural abundance of the soil microbial biomass. Soil Biology and Biochemistry 38(11):3257–3266. doi:10.1016/j.soilbio.2006.04.005

Ding X, He H, Zhang B, Zhang X (2011) Plant-N incorporation into microbial amino sugars as affected by inorganic N addition: A microcosm study of 15N-labeled maize residue decomposition. Soil Biology and Biochemistry 43(9):1968–1974. doi:10.1016/j.soilbio.2011.06.012

Ding Y, Watanabe A, Jaffé R (2014) Dissolved black nitrogen (DBN) in freshwater environments. Organic Geochemistry 68:1–4. doi:10.1016/j.orggeochem.2013.12.009

Dong W, Zhang X, Dai X, Fu X, Yang F, Liu X, Sun X, Wen X, Schaeffer S (2014) Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. Applied Soil Ecology 84:140–147. doi:10.1016/j.apsoil.2014.06.007

Engelking B, Flessa H, Joergensen RG (2007) Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil. Soil Biology and Biochemistry 39(8):2111–2118. doi:10.1016/j.soilbio.2007.03.020

Eusterhues K, Rennert T, Knicker H, Kögel-Knabner, I., Totsche, K.U., Schwertmann, U. (2011) Fractionation of organic matter due to reaction with ferrihydrite: coprecipitation versus adsorption. Environmental Science and Technology(45):527–533

Fang Y, Singh B, Singh BP, Krull E (2014) Biochar carbon stability in four contrasting soils. Eur J Soil Science 65(1):60–71. doi:10.1111/ejss.12094

Fitzpatrick RW, Schwertmann U (1982) Al-substituted goethite—An indicator of pedogenic and other weathering environments in South Africa. Geoderma(27):335–347

Frey SD, Elliott ET, Paustian K (1999) Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31(4):573–585. doi:10.1016/S0038-0717(98)00161-8

Friedel JK, Scheller E (2002) Composition of hydrolysable amino acids in soil organic matter and soil microbial biomass. Soil Biology and Biochemistry 34(3):315–325. doi:10.1016/S0038-0717(01)00185-7

Friedman M (1999) Chemistry, Nutrition, and Microbiology of d-Amino Acids. J. Agric. Food Chem. 47(9):3457–3479. doi:10.1021/jf990080u

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

Gadde B, Menke C, Wassmann R (2009) Rice straw as a renewable energy source in India, Thailand, and the Philippines: Overall potential and limitations for energy contribution and greenhouse gas mitigation. Biomass and Bioenergy 33(11):1532–1546. doi:10.1016/j.biombioe.2009.07.018

Gattinger A, Ruser R, Schloter M, Munch JC (2002) Microbial community structure varies in different soil zones of a potato field. J. Plant Nutr. Soil Sci. 165(4):421. doi:10.1002/1522-2624(200208)165:4<421:AID-JPLN421>3.0.CO;2-N

Geisseler D, Horwath WR, Joergensen RG, Ludwig B (2010) Pathways of nitrogen utilization by soil microorganisms – A review. Soil Biology and Biochemistry 42(12):2058–2067. doi:10.1016/j.soilbio.2010.08.021

Geisseler D, Horwath WR, Joergensen RG, Ludwig B (2010) Pathways of nitrogen utilization by soil microorganisms–a review. Soil Biology and Biochemistry 42(12):2058–2067

Gerin PA, Genet MJ, Herbillon AJ, Delvaux B (2003) Surface analysis of soil material by X-ray photoelectron spectroscopy. Eur J Soil Science 54(3):589–604. doi:10.1046/j.1365-2389.2003.00537.x

Giller KE (2001) Nitrogen fixation in tropical cropping systems. Cabi

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Organic Geochemistry 29(4):811–819. doi:10.1016/S0146-6380(98)00194-6

Glaser B, Turrión M-B, Alef K (2004) Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biology and Biochemistry 36(3):399–407. doi:10.1016/j.soilbio.2003.10.013

Gong Z (1983) Pedogenesis of paddy soil and its significance in soil classification. Soil Science 135(1)

Gonzalez Perez P, Zhang R, Wang X, Ye J, Huang D (2015) Characterization of the amino acid composition of soils under organic and conventional management after addition of different fertilizers. Journal of Soils and Sediments 15(4):890–901. doi:10.1007/s11368-014-1049-3

Gottselig N, Bol R, Nischwitz V, Vereecken H, Amelung W, Klumpp E (2014) Distribution of Phosphorus-Containing Fine Colloids and Nanoparticles in Stream Water of a Forest Catchment. Vadose Zone Journal 13(7):0. doi:10.2136/vzj2014.01.0005

Guggenberger G, Frey SD, Six J, Paustian K, Elliott ET (1999) Bacterial and Fungal Cell-Wall Residues in Conventional and No-Tillage Agroecosystems. Soil Science Society of America Journal 63(5):1188. doi:10.2136/sssaj1999.6351188x

Guo J, Hu X, Gao L, Xie K, Ling N, Shen Q, Hu S, Guo S (2017) The rice production practices of high yield and high nitrogen use efficiency in Jiangsu, China. Scientific reports 7(1):2101. doi:10.1038/s41598-017-02338-3

Haider K, Schäffer A (2009) Soil biochemistry. Science Publishers, Enfield, NH

Hammes K, Schmidt MWI, Smernik RJ, Currie LA, Ball WP, Nguyen TH, Louchouarn P, Houel S, Gustafsson Ö, Elmquist M, Cornelissen G, Skjemstad JO, Masiello CA, Song J, Peng P'a, Mitra S, Dunn JC, Hatcher PG, Hockaday WC, Smith DM, Hartkopf-Fröder C, Böhmer A, Lüer B, Huebert BJ, Amelung W, Brodowski S, Huang L, Zhang W, Gschwend PM, Flores-Cervantes DX, Largeau C, Rouzaud J-N, Rumpel C, Guggenberger G, Kaiser K, Rodionov A, Gonzalez-Vila FJ, Gonzalez-Perez JA, La Rosa JM de, Manning DAC, López-Capél E, Ding L (2007) Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. Global Biogeochem. Cycles 21(3):n/a-n/a. doi:10.1029/2006GB002914

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion. A review. Ann Microbiol 60(4):579–598. doi:10.1007/s13213-010-0117-1

Hesse R, Chassé T, Szargan R (2003) Unifit 2002--universal analysis software for photoelectron spectra. Analytical and bioanalytical chemistry 375(7):856–863. doi:10.1007/s00216-002-1705-0

Hill TR (1990) The effect of nitrogenous fertilizer and plant spacing on the yield of three Chinese vegetables — Kai lan, Tsoi sum and Pak choi. Scientia Horticulturae 45(1-2):11–20. doi:10.1016/0304-4238(90)90063-K

Hilscher A, Knicker H (2011) Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. Soil Biology and Biochemistry 43(2):261–270. doi:10.1016/j.soilbio.2010.10.007

Högberg MN, Bååth E, Nordgren A, Arnebrant K, Högberg P (2003) Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs - a hypothesis based on field observations in boreal forest. New Phytologist 160(1):225–238. doi:10.1046/j.1469-8137.2003.00867.x

Høgh-Jensen H, Schjoerring JK (1997) nteractions between white clover and ryegrass under contrasting nitrogen availability: N2 fixation, N fertilizer recovery, N transfer and water use efficiency. Plant Soil 197(2):187–199. doi:10.1023/A:1004289512040

Holland EA, Coleman DC (1987) Litter Placement Effects on Microbial and Organic Matter Dynamics in an Agroecosystem. Ecology 68(2):425–433. doi:10.2307/1939274

Houtermans M, Lehndorff E, Utami SR, Said-Pullicino D, Romani M, Kölbl A, Kaiser K, Cao ZH, Amelung W (2017) Nitrogen sequestration under long-term paddy management in soils developed on contrasting parent material. Biology and Fertility of Soils 53(8):837–848. doi:10.1007/s00374-017-1223-z

Hulugalle NR, Entwistle P (1997) Soil properties, nutrient uptake and crop growth in an irrigated Vertisol after nine years of minimum tillage. Soil and Tillage Research 42(1-2):15–32. doi:10.1016/S0167-1987(96)01104-X

Ingwersen J, Poll C, Streck T, Kandeler E (2008) Micro-scale modelling of carbon turnover driven by microbial succession at a biogeochemical interface. Soil Biology and Biochemistry 40(4):864–878. doi:10.1016/j.soilbio.2007.10.018

IUSS Working Group (Ed.) (2014) World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World soil resources reports, vol 106. FAO, Rome

Jiang X, Liu W, Liu Q, Jia Z, Wright AL, Cao Z (2013) Soil N mineralization, nitrification and dynamic changes in abundance of ammonia-oxidizing bacteria and archaea along a 2000 year chronosequence of rice cultivation. Plant and Soil 365(1-2):59–68

Joergensen RG, Wichern F (2008) Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40(12):2977–2991. doi:10.1016/j.soilbio.2008.08.017

Junet A de, Basile-Doelsch I, Borschneck D, Masion A, Legros S, Marol C, Balesdent J, Templier J, Derenne S (2013) Characterisation of organic matter from organo-mineral complexes in an Andosol from Reunion Island. Journal of Analytical and Applied Pyrolysis 99:92–100. doi:10.1016/j.jaap.2012.10.020

Kaiser K, Guggenberger G, Zech W (1996) Sorption of DOM and DOM fractions to forest soils. Geoderma 74(3-4):281–303. doi:10.1016/S0016-7061(96)00071-7

Kalbitz K, Kaiser K, Fiedler S, Kölbl A, Amelung W, Bräuer T, Cao Z, Don A, Grootes P, Jahn R, Schwark L, Vogelsang V, Wissing L, Kögel-Knabner I (2013) The carbon count of 2000 years of rice cultivation. Global change biology 19(4):1107–1113. doi:10.1111/gcb.12080

Kappenberg A, Bläsing M, Lehndorff E, Amelung W (2016) Black carbon assessment using benzene polycarboxylic acids: Limitations for organic-rich matrices. Organic Geochemistry 94:47–51. doi:10.1016/j.orggeochem.2016.01.009

Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environmental science & technology 44(4):1247–1253. doi:10.1021/es9031419

Khan KS, Mack R, Castillo X, Kaiser M, Joergensen RG (2016) Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271:115–123. doi:10.1016/j.geoderma.2016.02.019

Kimber RWL, Hare PE (1992) Wide range of racemization of amino acids in peptides from human fossil bone and its implications for amino acid racemization dating. Geochimica et Cosmochimica Acta 56(2):739–743

Kirk G (2004) The Biogeochemistry of Submerged Soils. John Wiley & Sons, Ltd, Chichester, UK

Kleber M, Mikutta R, Torn MS, Jahn R (2005) Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. European Journal of Soil Science 56(6):717–725

Knicker H (2007) How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85(1):91–118. doi:10.1007/s10533-007-9104-4

Kögel-Knabner I, Amelung W, Cao Z, Fiedler S, Frenzel P, Jahn R, Kalbitz K, Kölbl A, Schloter M (2010) Biogeochemistry of paddy soils. Geoderma 157(1-2):1–14. doi:10.1016/j.geoderma.2010.03.009

Kögel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils. Integrating biology, mineralogy, and organic matter chemistry. J. Plant Nutr. Soil Sci. 171(1):61–82. doi:10.1002/jpln.200700048

Kölbl A, Schad P, Jahn R, Amelung W, Bannert A, Cao ZH, Fiedler S, Kalbitz K, Lehndorff E, Müller-Niggemann C, Schloter M, Schwark L, Vogelsang V, Wissing L, Kögel-Knabner I (2014) Accelerated soil formation due to paddy management on marshlands (Zhejiang Province, China). Properties, processes and ecological functions of floodplain, peatland, and paddy soils 228–229:67–89. doi:10.1016/j.geoderma.2013.09.005

Kriszan M, Amelung W, Schellberg J, Gebbing T, Kühbauch W (2009) Long-term changes of the δ15N natural abundance of plants and soil in a temperate grassland. Plant Soil 325(1-2):157–169. doi:10.1007/s11104-009-9965-5

Kuzyakov Y, Bogomolova I, Glaser B (2014) Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific 14C analysis. Soil Biology and Biochemistry 70:229–236. doi:10.1016/j.soilbio.2013.12.021

Kvenvolden KA, Peterson E, Brown FS (1970) Racemization of amino acids in sediments from Saanich Inlet, British Columbia. Science 169(3950):1079–1082

La Rosa JM de, Knicker H (2011) Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43(12):2368–2373. doi:10.1016/j.soilbio.2011.08.008

Lehndorff E, Houtermans M, Winkler P, Kaiser K, Kölbl A, Romani M, Said-Pullicino D, Utami, SR, Zhang GL, Cao ZH (2016) Black carbon and black nitrogen storage under long-term paddy and non-paddy management in major reference soil groups. Geoderma 284:214–225

Lehndorff E, Brodowski S, Schmidt L, Haumaier L, Grootes PM, Rethemeyer J, Amelung W (2015) Industrial carbon input to arable soil since 1958. Organic Geochemistry 80:46–52. doi:10.1016/j.orggeochem.2015.01.003

Lehndorff E, Roth PJ, Cao Zh, Amelung W (2014) Black carbon accrual during 2000 years of paddy-rice and non-paddy cropping in the Yangtze River Delta, China. Global change biology 20(6):1968–1978. doi:10.1111/gcb.12468

Liang C, Gutknecht JLM, Balser TC (2015) Microbial lipid and amino sugar responses to long-term simulated global environmental changes in a California annual grassland. Frontiers in microbiology 6:385. doi:10.3389/fmicb.2015.00385

Liang C, Zhang X, Rubert KF, Balser TC (2007) Effect of plant materials on microbial transformation of amino sugars in three soil microcosms. Biology and Fertility of Soils 43(6):631–639. doi:10.1007/s00374-006-0142-1

Lima E, Boddey RM, Döbereiner J (1987) Quantification of biological nitrogen fixation associated with sugar cane using a 15 N aided nitrogen balance. Soil Biology and Biochemistry 19(2):165–170

Luetzow Mv, Kogel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils. Mechanisms and their relevance under different soil conditions - a review. Eur J Soil Science 57(4):426–445. doi:10.1111/j.1365-2389.2006.00809.x

Lützow MV, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. European Journal of Soil Science 57(4):426–445. doi:10.1111/j.1365-2389.2006.00809.x

Maclean JL, D Dawe, B Hardy, GP Hettel (ed) (2002) Rice almanac, Los Baños (Philippines), Bouaké (Côte d'Ivoire), Cali (Colombia) and Rome (Italy)

Major J, Lehmann J, Rondon M, Goodale C (2010) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Glob. Change Biol. 16(4):1366–1379. doi:10.1111/j.1365-2486.2009.02044.x

Maroudas A, Bayliss MT, Uchitel-Kaushansky N, Schneiderman R, Gilav E (1998) Aggrecan turnover in human articular cartilage: use of aspartic acid racemization as a marker of molecular age. Archives of biochemistry and biophysics 350(1):61–71

Martens DA, Loeffelmann KL (2003) Soil Amino Acid Composition Quantified by Acid Hydrolysis and Anion Chromatography−Pulsed Amperometry. J. Agric. Food Chem. 51(22):6521–6529. doi:10.1021/jf034422e

McBeath AV, Smernik RJ, Schneider MPW, Schmidt MWI, Plant EL (2011) Determination of the aromaticity and the degree of aromatic condensation of a thermosequence of wood charcoal using NMR. Organic Geochemistry 42(10):1194–1202. doi:10.1016/j.orggeochem.2011.08.008

McNeill AM, Wood M (1990) ^{15}N estimates of nitrogen fixation by white clover (Trifolium repens L.) growing in a mixture with ryegrass (Lolium perenne L.). Plant and Soil 128(2):265–273

Mejia J, Roden EE, Ginder-Vogel M (2016) Influence of Oxygen and Nitrate on Fe (Hydr)oxide Mineral Transformation and Soil Microbial Communities during Redox Cycling. Environmental science & technology 50(7):3580–3588. doi:10.1021/acs.est.5b05519

Mikutta R, Kaiser K (2011) Organic matter bound to mineral surfaces. Resistance to chemical and biological oxidation. Soil Biology and Biochemistry 43(8):1738–1741. doi:10.1016/j.soilbio.2011.04.012

Mikutta R, Kaiser K, Dörr N, Vollmer A, Chadwick OA, Chorover J, Kramer MG, Guggenberger G (2010) Mineralogical impact on organic nitrogen across a long-term soil chronosequence (0.3–4100kyr). Geochimica et Cosmochimica Acta 74(7):2142–2164

Mikutta R, Mikutta C, Kalbitz K, Scheel T, Kaiser K, Jahn R (2007) Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica et Cosmochimica Acta 71(10):2569–2590. doi:10.1016/j.gca.2007.03.002

Mikutta R, Schaumann GE, Gildemeister D, Bonneville S, Kramer MG, Chorover J, Chadwick OA, Guggenberger G (2009) Biogeochemistry of mineral–organic associations across a long-term mineralogical soil gradient (0.3–4100kyr), Hawaiian Islands. Geochimica et Cosmochimica Acta 73(7):2034–2060. doi:10.1016/j.gca.2008.12.028

Miltner A, Zech W (1999) Microbial degradation and resynthesis of proteins during incubation of beech leaf litter in the presence of mineral phases. Biology and Fertility of Soils 30(1-2):48–51. doi:10.1007/s003740050586

Nagata Y, Fujiwara T, Kawaguchi-Nagata K, Fukumori Y, Yamanaka T (1998) Occurrence of peptidyl D-amino acids in soluble fractions of several eubacteria, archaea and eukaryotes. Biochimica et Biophysica Acta (BBA)-General Subjects 1379(1):76–82

Nam JJ, Gustafsson O, Kurt-Karakus P, Breivik K, Steinnes E, Jones KC (2008) Relationships between organic matter, black carbon and persistent organic pollutants in European background soils: Implications for sources and environmental fate. Environmental pollution (Barking, Essex : 1987) 156(3):809–817. doi:10.1016/j.envpol.2008.05.027

Ofori F, Stern WR (1987) Cereal–Legume Intercropping Systems. In: Brady NC (ed) Advances in agronomy. Vol. 41, vol 41. Academic Press, San Diego, pp 41–90

Olk DC, Cassman KG, Randall EW, Kinchesh P, Sanger LJ, Anderson JM (1996) Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. European Journal of Soil Science 47(3):293–303. doi:10.1111/j.1365-2389.1996.tb01403.x

Pande KR, Becker M (2003) Seasonal soil nitrogen dynamics in rice-wheat cropping systems of Nepal. J. Plant Nutr. Soil Sci. 166(4):499–506. doi:10.1002/jpln.200320315

Parsons JW Chemistry and distribution of amino sugars in soils and soil organisms. Soil biochemistry 1981(5):197–227

Patra AK, Rego TJ (1997) Measurments of nitrate leaching potential of vertisol using bromide as tracer under rainfed conditions of the Indian semi-arid tropics. Soil Science 162(9):656–665

Pelz O, Cifuentes LA, Hammer BT, Kelley CA, Coffin RB (1998) Tracing the assimilation of organic compounds using δ13C analysis of unique amino acids in the bacterial peptidoglycan cell wall. FEMS Microbiology Ecology 25(3):229–240

Poinar HN, Höss M, Bada JL, Pääbo S (1996) Amino Acid Racemization and the Preservation of Ancient DNA. Science 272(5263):864–866. doi:10.1126/science.272.5263.864

Pronk GJ, Heister K, Kögel-Knabner I (2015) Amino sugars reflect microbial residues as affected by clay mineral composition of artificial soils. Organic Geochemistry 83-84:109–113. doi:10.1016/j.orggeochem.2015.03.007

Ren T, Wang J, Chen Q, Zhang F, Lu S (2014) The effects of manure and nitrogen fertilizer applications on soil organic carbon and nitrogen in a high-input cropping system. PloS one 9(5):e97732. doi:10.1371/journal.pone.0097732

Riedel T, Iden S, Geilich J, Wiedner K, Durner W, Biester H (2014) Changes in the molecular composition of organic matter leached from an agricultural topsoil following addition of biomass-derived black carbon (biochar). Organic Geochemistry 69:52–60. doi:10.1016/j.orggeochem.2014.02.003

Rodionov A, Amelung W, Peinemann N, Haumaier L, Zhang X, Kleber M, Glaser B, Urusevskaya I, Zech W (2010) Black carbon in grassland ecosystems of the world. Global Biogeochem. Cycles 24(3):n/a-n/a. doi:10.1029/2009GB003669

Roth PJ, Lehndorff E, Brodowski S, Bornemann L, Sanchez-García L, Gustafsson Ö, Amelung W (2012) Differentiation of charcoal, soot and diagenetic carbon in soil: Method comparison and perspectives. Organic Geochemistry 46:66–75. doi:10.1016/j.orggeochem.2012.01.012

Roth PJ, Lehndorff E, Cao Zh, Zhuang S, Bannert A, Wissing L, Schloter M, Kögel-Knabner I, Amelung W (2011) Accumulation of nitrogen and microbial residues during 2000 years of rice paddy and non-paddy soil development in the Yangtze River Delta, China. Glob. Change Biol. 17(11):3405–3417. doi:10.1111/j.1365-2486.2011.02500.x

Said-Pullicino D, Cucu MA, Sodano M, Birk JJ, Glaser B, Celi L (2014) Nitrogen immobilization in paddy soils as affected by redox conditions and rice straw incorporation. Properties, processes and ecological functions of floodplain, peatland, and paddy soils 228–229:44–53. doi:10.1016/j.geoderma.2013.06.020

Said-Pullicino D, Miniotti EF, Sodano M, Bertora C, Lerda C, Chiaradia, E.,A, Romani M, Cesari de Maria S, Sacco D, Celi L (2015) Linking dissolved organic carbon cycling to organic carbon fluxes in rice paddies under different water management practices. Plant and Soil:1–18

Schleifer KH, Kandler O (1970) Amino acid sequence of the murein of Planococcus and other Micrococcaceae. Journal of bacteriology 103(2):387–392

Schmidt-Rohr K, Mao J-D, Olk DC (2004) Nitrogen-bonded aromatics in soil organic matter and their implications for a yield decline in intensive rice cropping. Proceedings of the National Academy of Sciences of the United States of America 101(17):6351–6354

Schneider MPW, Hilf M, Vogt UF, Schmidt MWI (2010) The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200°C and 1000°C. Organic Geochemistry 41(10):1082–1088. doi:10.1016/j.orggeochem.2010.07.001

Schrumpf M, Kaiser K, Guggenberger G, Persson T, Kögel-Knabner I, Schulze E-D (2013) Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences(10):1675–1691

Schulten H-R, Schnitzer M (1997) The chemistry of soil organic nitrogen: a review. Biology and Fertility of Soils 26(1):1–15

Seiler W, Crutzen PJ (1980) Estimates of gross and net fluxes of carbon between the biosphere and the atmosphere from biomass burning. Climatic Change 2(3):207–247. doi:10.1007/BF00137988

Senwo NZ, Tabatabai AM (1998) Amino acid composition of soil organic matter. Biology and Fertility of Soils 26(3):235–242. doi:10.1007/s003740050373

Simpson RT, Frey SD, Six J, Thiet RK (2004) Preferential Accumulation of Microbial Carbon in Aggregate Structures of No-Tillage Soils. Soil Science Society of America Journal 68(4):1249. doi:10.2136/sssaj2004.1249

Singh BP, Cowie AL, Smernik RJ (2012) Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. Environmental science & technology 46(21):11770–11778. doi:10.1021/es302545b

Singh N, Abiven S, Torn MS, Schmidt MWI (2012) Fire-derived organic carbon in soil turns over on a centennial scale. Biogeosciences 9(8):2847–2857. doi:10.5194/bg-9-2847-2012

Six J, Paustian K, Elliott ET, Combrink C (2000) Soil Structure and Organic Matter. Soil Science Society of America Journal 64(2):681. doi:10.2136/sssaj2000.642681x

Steinbach A, Schulz S, Giebler J, Pronk GJ, Kögel-Knabner I, Harms H, Wick LY, Schloter M (2015) Clay minerals and metal oxides strongly influence the structure of alkane-degrading microbial communities during soil maturation. The ISME journal 9(7):1687–1691. doi:10.1038/ismej.2014.243

Stevenson FJ (1982) Nitrogen in agricultural soils. American Society of Agronomy

Tong C, Xiao H, Tang G, Wang H, Huang T, Xia H, Keith SJ, Li Y, Liu S, Wu J (2009) Long-term fertilizer effects on organic carbon and total nitrogen and coupling relationships of C and N in paddy soils in subtropical China. Soil and Tillage Research 106(1):8–14

Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. Nature 389(6647):170–173. doi:10.1038/38260

Van Kessel C, Farrell RE, Roskoski JP, Keane KM (1994) Recycling of the naturally-occurring 15N In an established stand of Leucaena leucocephala. Soil Biology and Biochemistry 26(6):757–762. doi:10.1016/0038-0717(94)90269-0

Wada K (1985) The distinctive properties of Andosols. In: Advances in soil science. Springer, pp 173–229

Wang L, Xue C, Pan X, Chen F, Liu Y (2018) Application of Controlled-Release Urea Enhances Grain Yield and Nitrogen Use Efficiency in Irrigated Rice in the Yangtze River Basin, China. Frontiers in plant science 9:999. doi:10.3389/fpls.2018.00999

Ward CP, Sleighter RL, Hatcher PG, Cory RM (2014) Insights into the complete and partial photooxidation of black carbon in surface waters. Environmental science. Processes & impacts 16(4):721–731. doi:10.1039/c3em00597f

Watzka M, Buchgraber K, Wanek W (2006) Natural 15N abundance of plants and soils under different management practices in a montane grassland. Soil Biology and Biochemistry 38(7):1564–1576. doi:10.1016/j.soilbio.2005.11.007

Webley DM, Jones D (1971) Biological transformation of microbial residues in soil. Soil biochemistry(2):S. 446–485

West DW, Black JDF (1978) Irrigation timing-its influnence on the effects of salinity and waterlogging stresses in tobacco plants. Soil Science 125(6):367–376. doi:10.1097/00010694-197806000-00005

Winkler P, Kaiser K, Kölbl A, Kühn T, Schad P, Urbanski L, Fiedler S, Lehndorff E, Kalbitz K, Utami SR, Cao Z, Zhang G, Jahn R, Kögel-Knabner I (2016) Response of Vertisols, Andosols, and Alisols to paddy management. Geoderma 261:23–35. doi:10.1016/j.geoderma.2015.06.017

Wissing L, Kölbl A, Vogelsang V, Fu J-R, Cao Z-H, Kögel-Knabner I (2011) Organic carbon accumulation in a 2000-year chronosequence of paddy soil evolution. CATENA 87(3):376–385

Witt C, Cassman KG, Ottow JCG, Biker U (1998) Soil microbial biomass and nitrogen supply in an irrigated lowland rice soil as affected by crop rotation and residue management. Biology and Fertility of Soils 28(1):71–80. doi:10.1007/s003740050465

Wolf M, Lehndorff E, Wiesenberg GLB, Stockhausen M, Schwark L, Amelung W (2013) Towards reconstruction of past fire regimes from geochemical analysis of charcoal. Organic Geochemistry 55:11–21. doi:10.1016/j.orggeochem.2012.11.002

Yamaguchi NU, Okazaki M (2002) Micro volume changes due to Pb(II) and Cu(II) sorption on amorphous Fe(III) hydroxide. Journal of colloid and interface science 249(2):489–491. doi:10.1006/jcis.2002.8276

Yamamoto K, Ohtani S (1991) Age estimation using the racemization of amino acid in human dentin. Journal of Forensic Science 36(3):792–800

Yoneyama T, Kouno K, Yazaki J (1990) Variation of natural 15 N abundance of crops and soils in Japan with special reference to the effect of soil conditions and Fertilizer application. Soil Science and Plant Nutrition 36(4):667–675. doi:10.1080/00380768.1990.10416804

Zech W, Schad P, Hintermaier-Erhard G (2014) Böden der Welt: ein Bildatlas. Springer-Verlag

Zhang G-L, Gong Z-T (2003) Pedogenic evolution of paddy soils in different soil landscapes. Anthropogenic soils and their use in China: Anthropogenic soils and soil quality change under intensive management in China 115(1–2):15–29. doi:10.1016/S0016-7061(03)00072-7

Zhang W, Cui Y, Lu X, Bai E, He H, Xie H, Liang C, Zhang X (2016) High nitrogen deposition decreases the contribution of fungal residues to soil carbon pools in a tropical forest ecosystem. Soil Biology and Biochemistry 97:211–214. doi:10.1016/j.soilbio.2016.03.019

Zhang X, Amelung W (1996) Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. Soil Biology and Biochemistry 28(9):1201–1206. doi:10.1016/0038-0717(96)00117-4

Zhao X, Xie Y-X, Xiong Z-Q, Yan X-Y, Xing G-X, Zhu Z-L (2009) Nitrogen fate and environmental consequence in paddy soil under rice-wheat rotation in the Taihu lake region, China. Plant and Soil 319(1):225–234. doi:10.1007/s11104-008-9865-0

Zimmermann M, Bird MI, Wurster C, Saiz G, Goodrick I, Barta J, Capek P, Santruckova H, Smernik R (2012) Rapid degradation of pyrogenic carbon. Glob. Change Biol. 18(11):3306–3316. doi:10.1111/j.1365-2486.2012.02796.x

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