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

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Abstract:

Long-term paddy management promotes nitrogen (N) sequestration, but it is unknown to what extent the properties of the parent soil modify the management-induced N sequestration in peptide-bound amino acids (AA-N). We hypothesized that paddy management effects on the storage of AA-N relate to the mineral assembly. Hence, we determined contents and chirality of peptide-bound amino acids in paddy soils developed on contrasting parent material (Vertisols, Andosols, Alisols in Indonesia, Alisols in China, and a Gleysol/Fluvisol in Italy). Adjacent non-paddy soils served as references. Selected 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 proportions of N and AA-N, possibly because N fixing intercrops masked the paddy management effects on N sequestration processes. Among the different soils developed on contrasting parent material, AA-N stocks were largest in Andosols, followed by Alisols and Fluvisols, and lowest in Vertisols. The N storage in amino acid forms went along with elevated D-contents of bacteria-derived alanine and glutamic acid, as well as with increasing stocks of DCB-extractable Fe, Mn and Al. Other D-amino acids, likely formed by racemization processes, did not vary systematically between paddy and non-paddy managed soils. Our data suggest that the presence of oxides increase the N sequestration in peptide-bound amino acids after microbial N transformations.

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 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).

Material and methods

Sampling

We sampled five soils developed on contrasting parent material under paddy and adjacent non-paddy management, 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 (Table 1), 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) (Table 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; Table S1). In addition, we took undisturbed soil cores (100 cm³, 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.

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 CO₂ (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 (\approx 30-40 and \approx 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 μ m). 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.

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. 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. 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 significantly 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. S2 a,b, Supplementary Materials). No clear relations of N to other soil properties such as texture, CEC, or pH were found.

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. 1), and so were the contributions of amino acids to total N (Fig. 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. 2). In general, the soils with the largest N stocks also tended to have the largest AA-N stocks (Fig. 1,2).

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. S1; Supplementary Materials) 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. S2). Therefore, we have to refrain from relating overall N sequestration in amino acids to single soil group specific properties.

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. 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. 3). The amino acid composition in the other four soil groups remained fairly similar.

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 (Table S1; Supplementary Materials). 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. 4; for lysine data see Table S1, Supplementary Materials). 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 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. 5 a). 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. 5 c). This suggests that higher D-enantiomer contributions of alanine occurred in oxide-rich soil. We also found correlations between D-/L- ratios of alanine to Fe_D and Al_D stocks ($R^2 = 0.45-0.53$, respectively; $P < 0.05$, Fig. S2 g,h; Supplementary Materials). 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 (Table S1; Supplementary Materials).

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. 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. 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. 6). The losses of AA-N were smallest for the Al_D -poor Fluvisol and Vertisols, and largest for the Al_D -rich Andosols.

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 ($P < 0.05$; Table 1, Fig. 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. 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. 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. 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. 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. 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 hydrolysed 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. 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. 4), while C/N ratios remained rather uniform throughout the depth profiles except for the Chinese Alisols where 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. S2 h; Supplementary Materials). 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. 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. Aluminium 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 (Figure S4, Supplementary Materials). 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 (Figs. 4 and 5 a).

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 peptidoglycane, 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.

Acknowledgements

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Supporting materials

We provide additional data and figures as supplementary material. In Table S1 D/L ratios of alanine, aspartic acid and lysine are presented. In Figure 1 the Fe_D, Al_D and Mn_D stocks in paddy and non-paddy in top- and subsoils of five major reference soil groups, in Figure S2 the relationships between dithionite extractable Fe_D and Al_D stocks and the stocks of N_t, amino acid-N, and the D/L ratio of alanine and glutamic acid, and in Figure S3 the correlation of Fe_D and Al_D stocks indicates isomorphic exchange of Fe with Al in soil oxides are presented.

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Table and figure captions

Table 1: Soil classification, sampling location, climate and crop history for Indonesia, China and Italy.

Figure 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.

Figure 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.

Figure 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.

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

Figure 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.

Figure 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.

Tab.4
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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)	volcanic ash, intermediate igneous (andesite)	silt-clay loam	2000-4400 / tropical (25°C)	<i>Oryza sativa</i> (rice), <i>Oryza sativa</i> , <i>Brassica rapa chinensis</i> (pak choi)	870-871	S 06°52.802' / E 106°56.457'
Silandic Andosol (Distric, Thixotropic)	Sukabumi				<i>Manihot esculenta</i> (cassava), <i>Zea maya</i> (maize)	969-981	S 06°52.029' / E 106°56.725'
Alisol-derived paddy Escalic Hydragrick Anthrosol (Dystric, Clayic)	Indonesia (Java)	andesite	silt loam	2500-3500 / tropical (27°C)	<i>Oryza sativa</i> (rice), <i>Oryza sativa</i> , <i>Zea maya</i> (maize)	238-248	S 06°32.205' / E 106°31.062'
Hyperalic Alisol (Abruptic, Humic, Clayic, Chromic)	Jasinga				agroforest: <i>Manihot esculenta</i> (cassava), <i>Musa spec.</i> (banana), <i>Leucaena leucocephala</i> (mimosa)	236-238	S 06°32.158' / E 106°31.030'
Vertisol-derived paddy Hydragrick Anthrosol (Eutric, Clayic)	Indonesia (Java)	Fluvial sediments from andesite	clay-silt clay	2000-3000 / tropical (27°C)	<i>Oryza sativa</i> (rice), <i>Oryza sativa</i> , <i>Nicotiana spec.</i> (tobacco)	77-78	S 07°26.878' / E 111°36.599'
Pisocalcic Mollic Grumic Vertisol (Humic, Hypereutric, Pellic)	Ngawi				<i>Saccharum officinarum</i> (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)	<i>Oryza sativa</i> (rice), <i>Trifolium spec.</i> (clover)	40-45	N 28°14.020' / E 116°53.866'
Cutanic Hyperalic Alisol (Chromic)					<i>Arachis hypogaea</i> (peanuts), <i>Sesamum indicum</i> (sesame), <i>Ipomoea batatas</i> (sweet potatoes)	50-57	N 28°14.035' / E 116°53.784'
Fluvisol-derived paddy Haplic Gleysol (<i>Eutric</i> , <i>Arenic</i> , <i>Densic</i>)	Italy, Zeme	alluvial sediment	sandy loam	700-1000 / temperate (13 °C)	<i>Oryza sativa</i> (rice), fallow	79-80	N 45°11.536' / E 8°40.078'
Endogleyic Fluvisol (<i>Eutric Arenic</i>)					<i>Zea maya</i> (maize), fallow	79-80	N 45°11.555' / E 8°40.104'

Fig. 21
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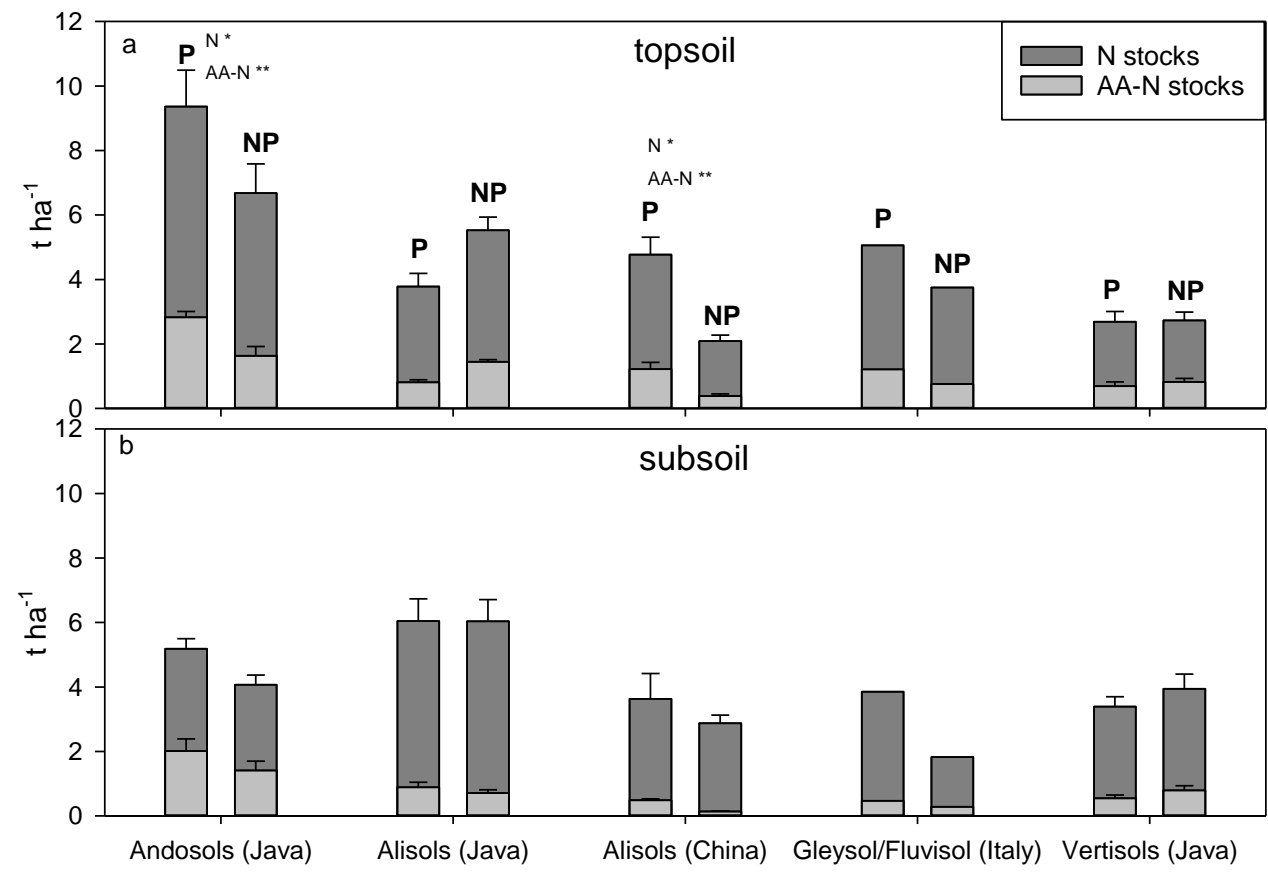
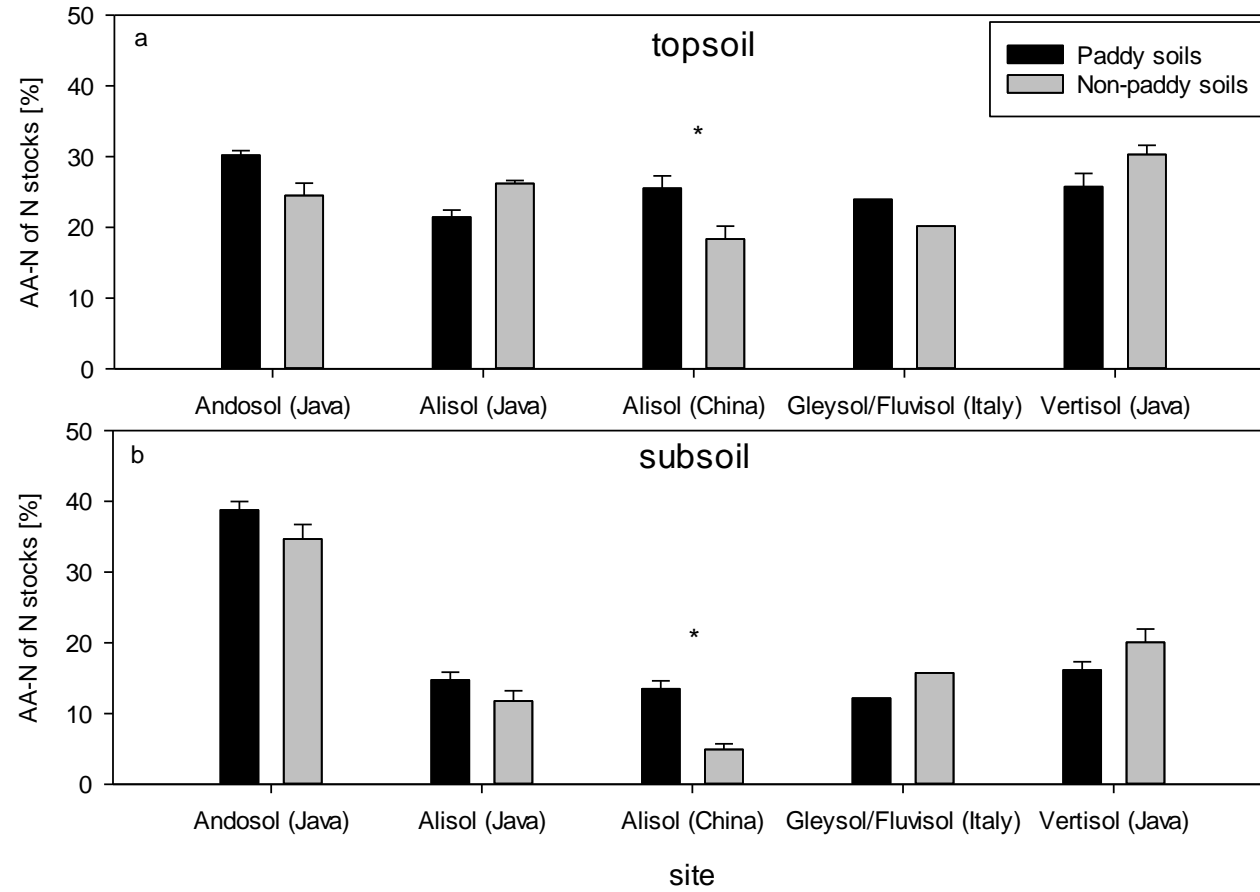


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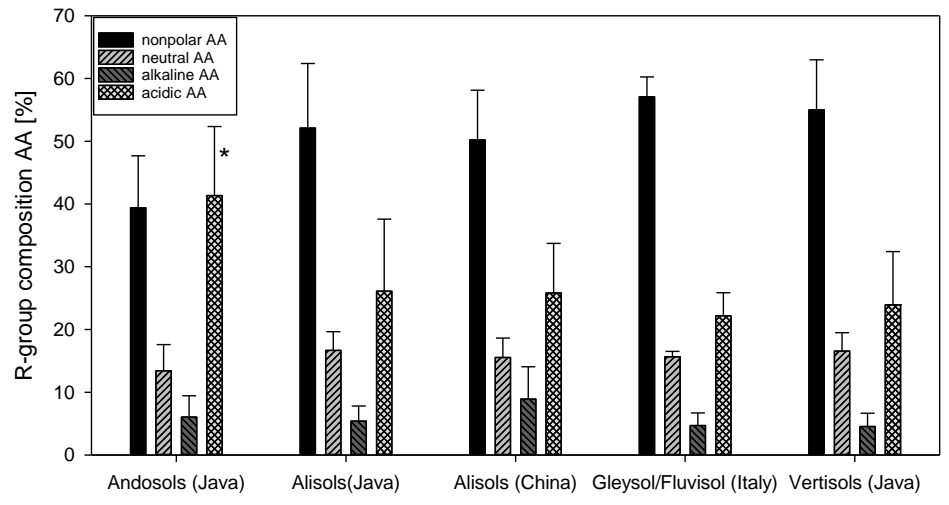
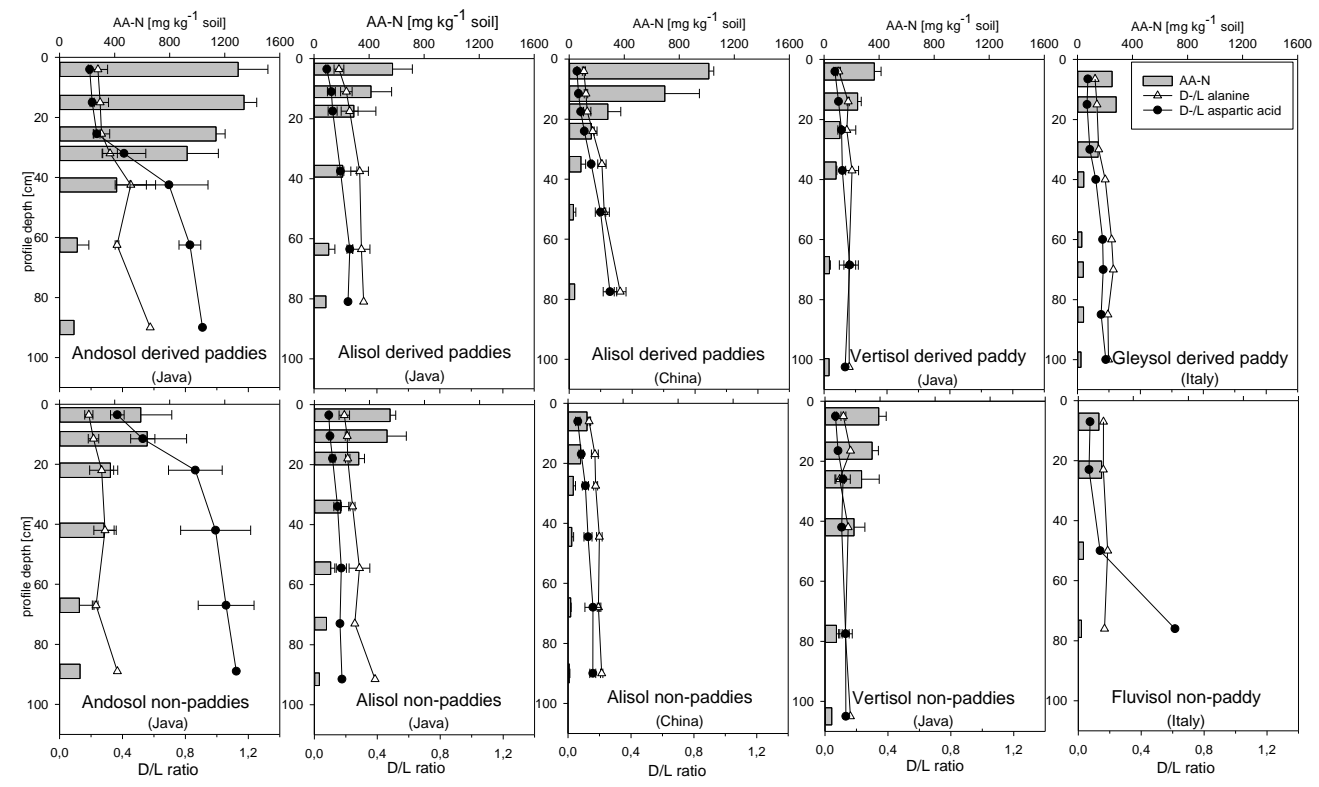


Fig. 4

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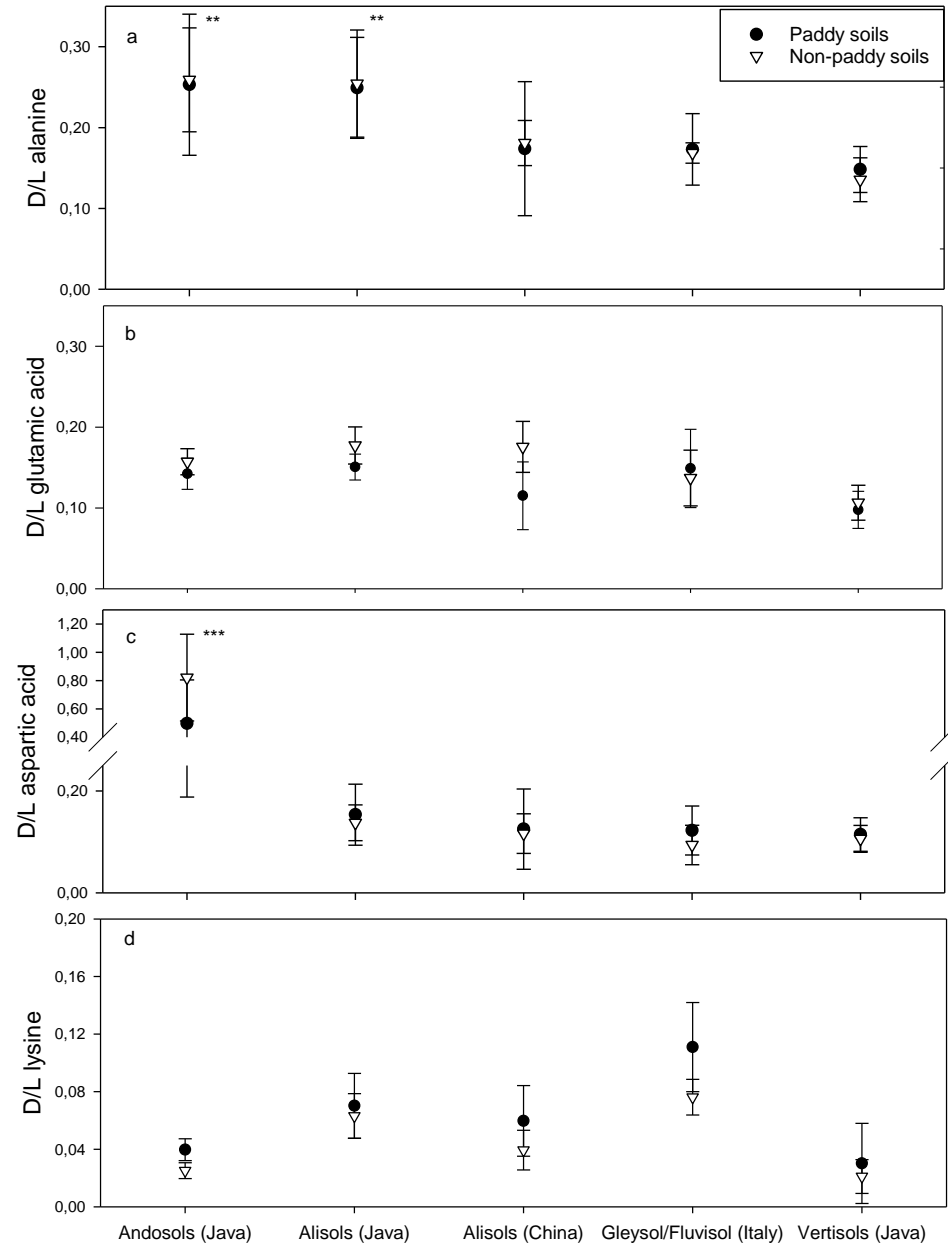


Fig. 6
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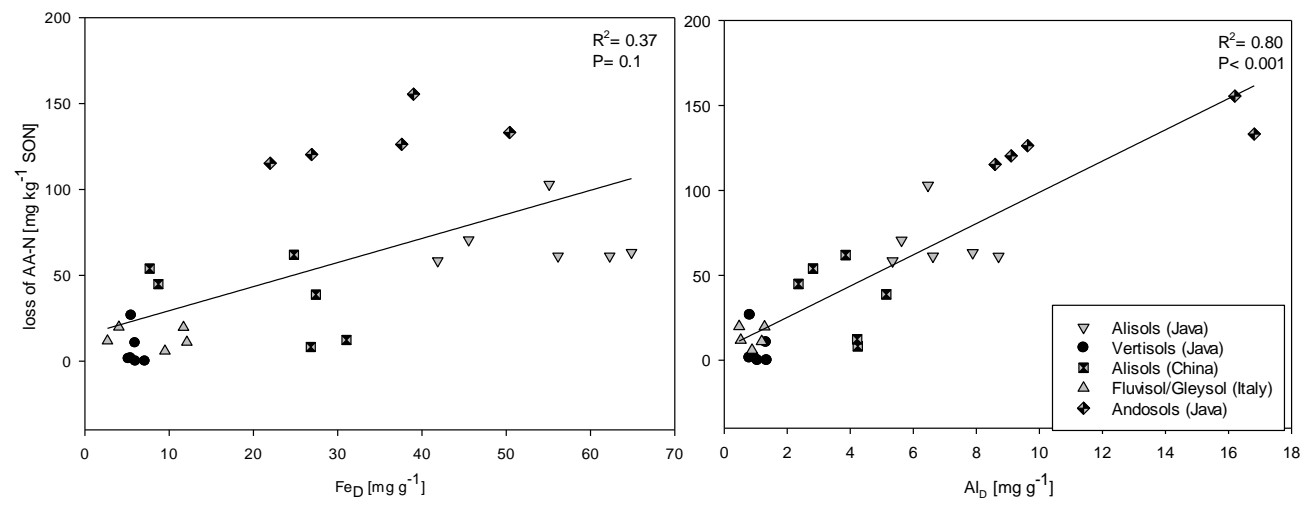


Table S1: D/L ratios of alanine, glutamic acid, aspartic acid and lysine; n= number of field replicates, *= mean of one field replicate and two interpolated values.

site	n	Paddies					Non Paddies				
		soil depth [cm]	Alanine	Glutamic acid	Aspartic acid	Lysine	soil depth [cm]	Alanine	Glutamic acid	Aspartic 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	0.26	0.10						
Fluvisol Gleysol (Italy)	1	0-13	0.11	0.09	0.07	0.09	0-15	0.16	0.14	0.07	0.07
	1	13-25	0.12	0.08	0.06	0.06	15-30	0.16	0.13	0.07	0.06
	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	

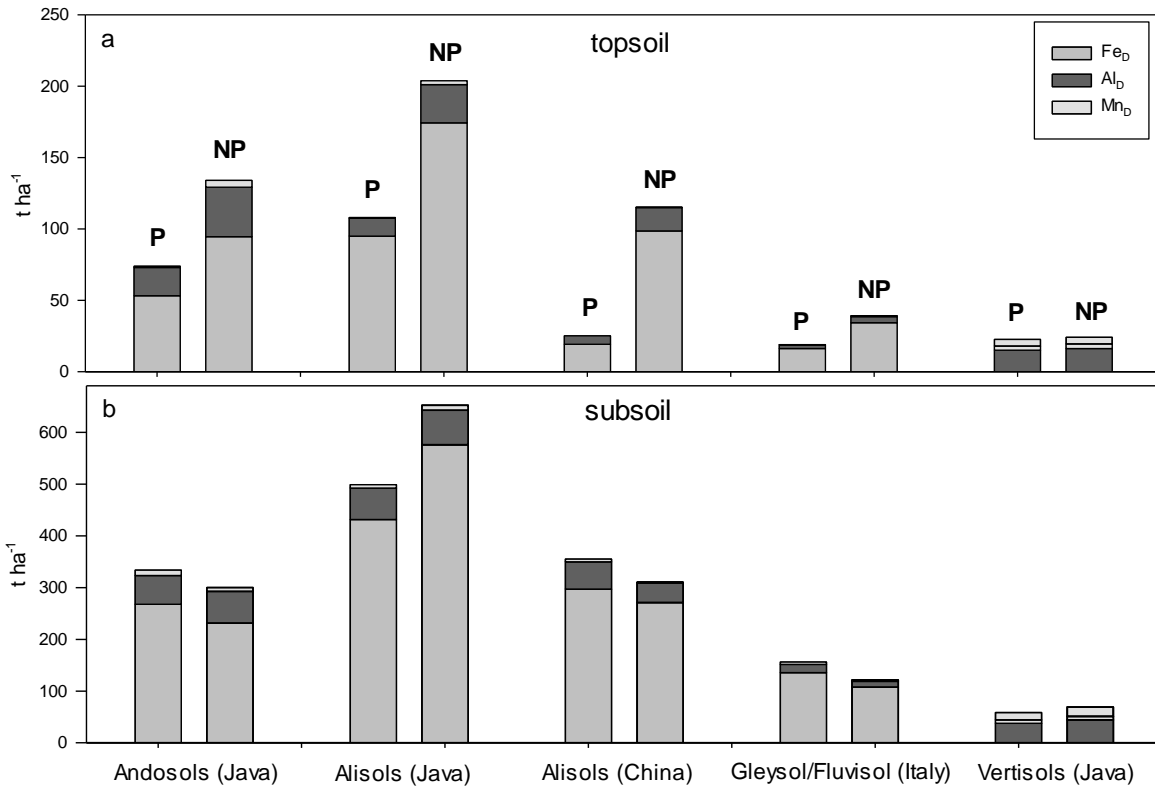


Figure S1 a,b: Dithionite-extractable Fe_D, Al_D and Mn_D stocks in paddy (P) and non-paddy (NP) in (a) top- and (b) subsoils of five major reference soil groups (3 field replicates, except for Italian site); data taken from Winkler et al., 2016).

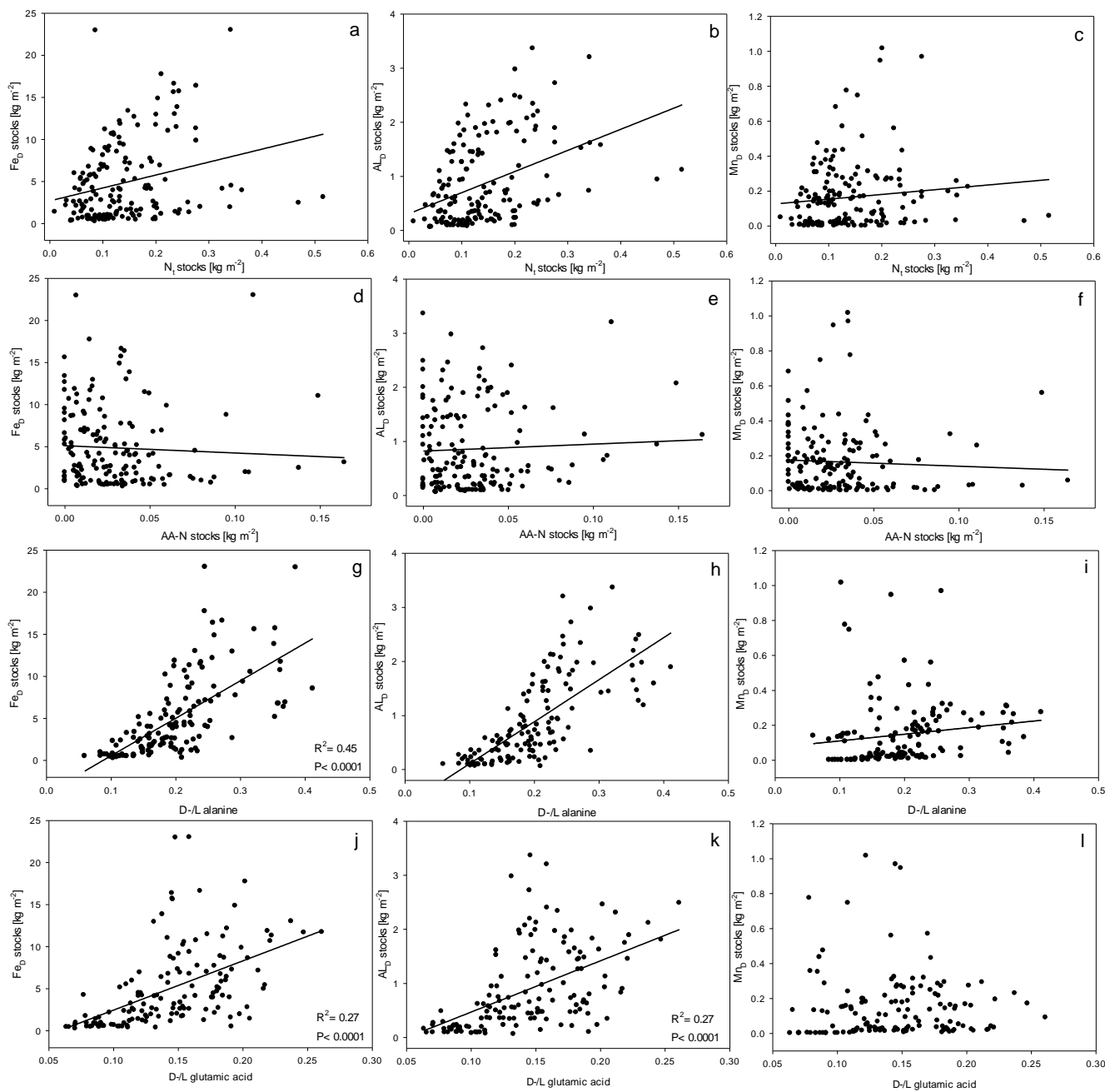


Figure S2: Relationships between dithionite extractable Fe_D, Al_D and Mn_D stocks and the stocks of N_t, AA-N, and the D/L ratio of alanine and glutamic acid.

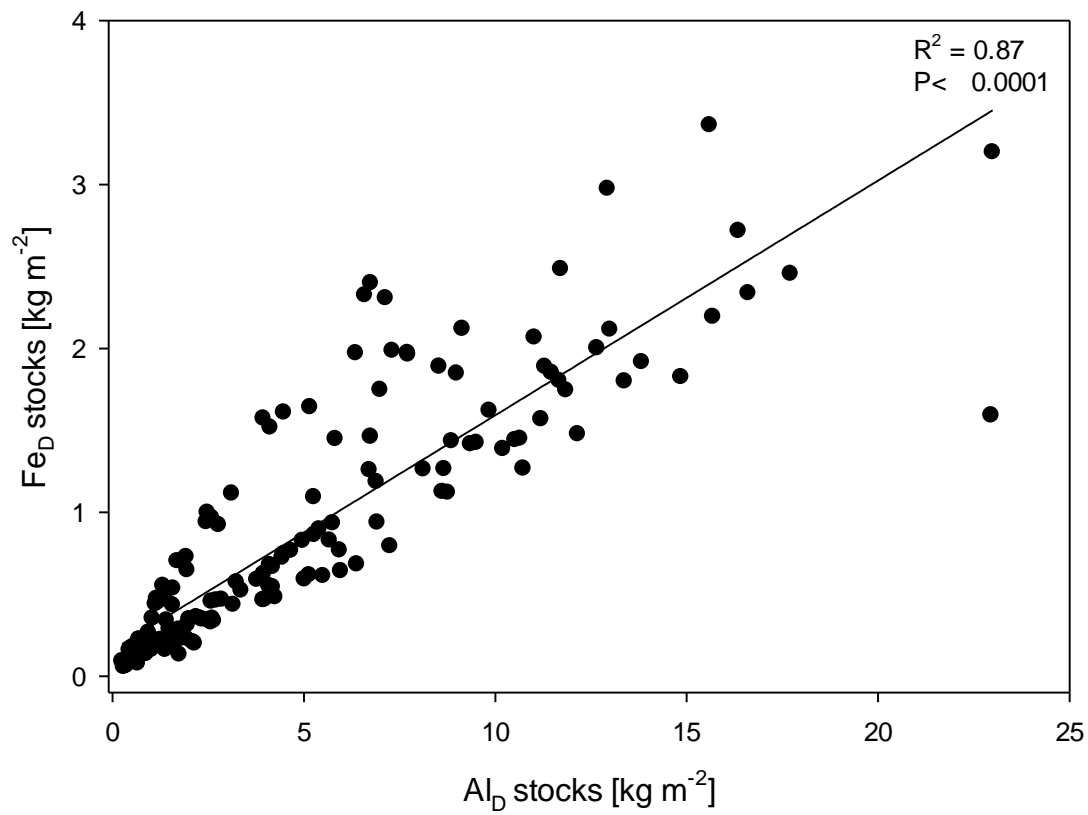


Figure S3: Correlation between dithionite-extractable Fe_D and Al_D indicates atomic substitution exchange of Fe for Al in soil oxides.