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Losses and biogeochemical cycling of soil organic nitrogen with prolonged arable cropping in the South African Highveld – evidence from D- and L-amino acids

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Abstract. We know little about the mechanisms that cause rapid losses in the soil organic N pool during cropping. As the analysis of amino acid enantiomers can provide insight into both the fate of microbial N and the ageing of cells in the environment, we used this technique as a tool to examine how the pool of protein-bound N in subtropical Plinthosols responds to increasing duration of arable cropping. The samples comprised bulk soils (0–20 cm) and clay fractions from each of three agro-ecosystems in semiarid South Africa; the sites have been cropped for periods varying from 0 to 98 years. The amino acid enantiomers contributed 34% to the total N content. With increasing number of years a piece of land has been cropped, the amino acid concentrations declined bi-exponentially to about 30% of their initial level in the native grasslands. Changes of the remaining soil protein-N pool were indicated by alterations in the D-content of individual amino acids. As the years of arable cropping increased, the proportions of D-alanine and D-glutamic acid increased relative to the respective L-enantiomers. This was attributed to an accumulation of N in residues of bacterial cell walls. In contrast, the D/L-ratios of leucine and aspartic acid declined in the long-term cultivated plots, probably reflecting losses of old amino acid-N reserves at the most degraded arable land.

Introduction

Nitrogen is the most common limiting macronutrient in crop production (Jenkinson 1981). To ensure sustainable N supply for plants, it is therefore necessary to understand the effects of cropping duration on soil N reservoirs (Paustian et al. 1997). Such understanding is especially important for the coarse-textured soils of South Africa with low N-supply capacities compared to other soils in tropical and subtropical climates (Du Toit et al. 1994; Du Preez and Du Toit 1995; Feller and Beare 1997). As in other regions the semiarid ecosystems loose N rapidly upon prolonged cropping (Dalal and Mayer 1987; Lobe et al. 2001).

As protein-bound nitrogen builds up a major proportion of N in soils (Sowden 1977; Stevenson 1982; Knicker and Kögel-Knabner 1998), analysis of amino acids

may provide a useful tool in elucidating soil N transformations in arable land (e.g., Dormaar 1983; Campbell et al. 1991; Szajdak et al. 2003). To obtain pools of different soil organic matter (SOM) accessibility, physical separation of soil particle-size fractions has been recommended (Christensen 1992). These fractions generally differed with respect to their SOM age (Scharpenseel et al. 1986), turnover rate (Dalal and Mayer 1987; Buyanovski et al. 1994; Christensen 1996), and origin (Guggenberger et al. 1994; Leinweber 1995). Especially the clay fraction has been found to be enriched with microbe-derived SOM (Kandeler et al. 2000; Amelung et al. 2002). Nevertheless, the origin and age of amino acids are difficult to assess.

Many amino acids occur in an optically active form. Living cells of microorganisms and plants mainly consist of L-enantiomers, while there are mainly three origins of D-enantiomers. First, certain D-amino acids such as D-alanine and D-glutamic acid are part of the bacterial cell wall (Weidel and Pelzer 1964; Voet and Voet 1995), thus serving as potential indicators of bacterial residues in soils (Pelz et al. 1998) and surface waters (Dittmar et al. 2001). Second, D-amino acids occur in antibiotics and antimicrobial peptides (Kuhn and Sommerville 1971; Mignogna et al. 1998) and third, D-amino acids may be converted from the L- to the D-form during cell ageing (Bada 1985). Given that the latter process prevails, the D/L-ratios of amino acids may be used for the determination of cell ageing (Kvenvolden and Peterson 1970; Mahaney and Rutter 1989; Lubec and Lubec 1993). While absolute age assessment on the basis of the racemization of amino acids frequently failed due to microbial alteration of remnant proteins and the hardly predictable effects of catalysts, environmental factors and protein structure on racemization rates (Bada 1985; Liardon and Ledermann 1986; Smith and de Sol 1986), relative protein dating on the basis of different D/L ratios of aspartic acid (Harada et al. 1996; Amelung 2001) or of other rare amino acids (e.g., proline, Kimber et al. 1990) has been suggested. Yet, possibly due to the lack of sophisticated analysis, effects of cultivation on soil organic N age as revealed by amino acid D/L ratios remained unclear (Griffin and Kimber 1988).

The objective of this study was to use amino acid-enantiomer assessment to gain for the very first time an insight into the origin and relative age of soil organic N as it is affected by the duration of long-term arable cropping of South African Plinthosols.

Materials and methods

Samples

Composite samples (0–20 cm) were taken from eight sites with different cropping periods (2–98 years) and an adjacent permanent native grassland in June/July 1998 in each of three agro-ecosystems near Harrismith (HS), Kroonstad (KR) and Tweespruit (TW), in the Free State Province of South Africa (Table 1; Figure 1). The cropped sites were subjected to similar farming practices, that is, regular ploughing to a depth of 20–30 cm (except for the 30- and 68-year-old fields in

Table 1. Climatic data and soil properties of the three agro-ecosystems at Harrismith, Kroonstad, and Tweespruit (data from Lobe et al. 2001).

Area	Clay (%)	MDC (years)	MAT (°C)	MAP (mm)	pH (H_2O)	pH (KCl)	CEC_{pot} ($mmol_c\,kg^{-1}$)
Harrismith	13–19	90	13.8	625	4.56–5.72	3.76–4.61	63–130
Kroonstad	10–15	98	16.6	563	5.24–6.82	4.02–5.53	42–84
Tweespruit	10–16	90	16.0	516	5.35–6.30	4.21–5.15	49–120

MDC = maximum duration of cultivation, MAT = mean annual temperature, MAP = mean annual precipitation, CEC_{pot} = potential cation exchange capacity.

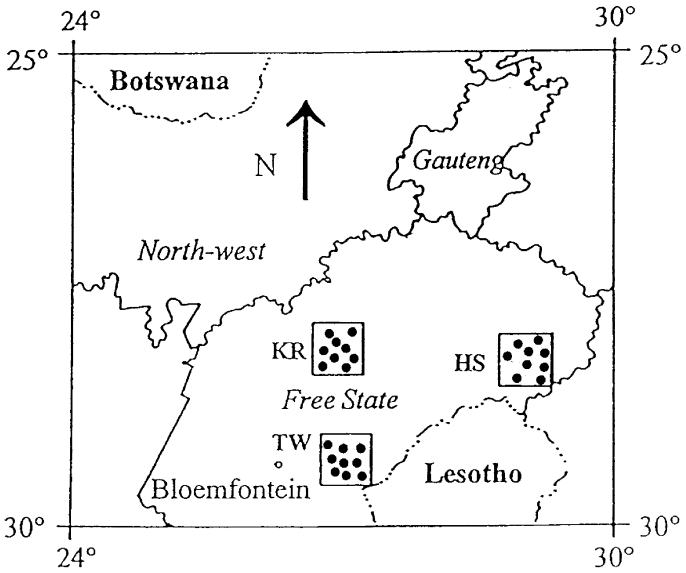

Figure 1. Locality of the sites under study (Lobe et al. 2001).

Harrismith which were ploughed to 40 cm), no irrigation (except for the 2-year-old field in Tweespruit, which was irrigated), a crop rotation between wheat (*Triticum aestivum*), maize (*Zea mays*), and sunflower (*Helianthus* sp.), and regular application of inorganic fertilizers (maize: 50–70 kg N/10–25 kg P/0–10 kg K ha^{-1}; wheat: 10–40 kg N/10–25 kg P/0–15 kg K ha^{-1}; sunflower: 20–50 kg N/10–20 kg P/2–6 kg K ha^{-1}). The grassland was used as grazing for cattle or sheep and was never ploughed. Details were described by Lobe et al. (2001; see also there for C and N contents, and Amelung et al. 2002 for amino sugar data). The soils were classified as Plinthustalfs according to Soil Taxonomy (Soil Survey Staff 1998), and as Plinthosols according to FAO (FAO 1997). Average grain yields varied from 2.2–3.8 t ha^{-1} for maize, from 1.2 to 2.8 t ha^{-1} for wheat, and from 1.0 to 1.3 t ha^{-1} for sunflower. All samples were air-dried and sieved to <2 mm prior to fractionation and chemical analysis.

Because of compaction of the arable land (bulk density $= 1.43\,g\,cm^{-3} \pm 0.12\,g\,cm^{-3}$, not being correlated with the duration of cropping), sampling by 0–20 cm depth intervals yields about 4% (corresponding to one additional depth cm) more subsurface material from the cropped fields than from the native grassland (bulk density $= 1.38\,g\,cm^{-3} \pm 0.11\,g\,cm^{-3}$). As the savanna subsoil contained less C and N than the topsoil, sampling by depth thus included the risk of overestimating storage of SOM in the savanna topsoil. To avoid an overestimation of amino acid loss rates due to arable cropping, amino acid concentrations in the grassland were corrected for bulk density measurements and element contents in the subsoil material. On doing so, we assumed that the C-normalized amino acid concentrations in the 0–21 cm depth interval were not significantly different from the sampled 0–20 cm depth interval at the pastures. Thus correcting for bulk density differences at the pasture sites resulted in a reduction of 1.3% in amino acid concentrations relative to the uncorrected ones.

Particle-size fractions

Particle-size fractionation was carried out by Lobe et al. (2001). Amino acids were determined in the bulk soil and the clay fraction ($<2\,\mu m$).

Our soils contained a maximum of 15% clay with kaolinite as major clay mineral (Land Type Survey Staff 2001). Hence, there was little if any fixed NH^{4+} remaining in the clay fraction, and we may consider total N as being mainly organic N.

Chemical analysis

Kvenvolden and Peterson (1970) suggested to remove free amino acids from soil with 1 M HCl. Preliminary experiments revealed that the amount of D- and L-amino acids occurring in free form was insignificant for the soils under study, amounting to $\leq 2\%$ of the total amino acid content. Also after particle-size fractionation, more than 99% of the amino acid enantiomers were recovered, suggesting that little if any amino acids were removed with the fractionation water, and confirming that the concentration of free amino acids was negligibly small in these soils. These results are confirmed by the findings of Sowden and Ivarson (1966) that free amino acids extracted by H_2O even in the presence of CCl_4 made up less than 1% of the total amino acid content recovered from soil. Thus, free amino acids were not routinely removed from the Plinthosols.

Determination of amino acid enantiomers followed the outline of Amelung and Zhang (2001). Briefly, the amino acids were hydrolyzed with 6 M HCl (12 h, 105 °C), purified via cation exchange resins utilizing oxalate rinsing for metal removal, and converted to *N*-pentafluoropropionyl-amino acid isopropyl esters. The esters were then separated on a chiral capillary column using gas chromatography, followed by flame ionization detection. Peak identity was confirmed by a Hewlett Packard Model 5890 gas chromatograph/mass spectrometer (Hewlett-Packard, Palo Alto, CA,

USA) with electron impact ionization. Quantification was performed relative to L-norvaline added to the samples after hydrolysis. D-methionine was used as second internal standard to determine the recovery of the L-norvaline. After method performance, 100±16% of L-norvaline were recovered, on the average.

Acid hydrolysis converts glutamine to glutamic acid and asparagine to aspartic acid. Nevertheless, in the following we only refer to the acidic amino acids, as these were the only respective derivatives identified in the GC chromatograms. D-phenylalanine and D-tyrosine were not interpreted as we could not discount the possibility that respective peaks were interfered with impurities in the FID chromatogram (Amelung and Zhang 2001). We also did not consider the D-forms of serine due to the strong liner sensitivity of this compound (Amelung and Zhang 2001).

Method-induced racemization

There is a risk that amino acids racemize during hydrolysis. During this reaction, the hydrogen bound to the α-carbon atom is removed and another proton is added from the reverse side (Carey and Sundberg 1995). Performing hydrolysis in a deuterated medium therefore leads to a direct labeling of the inverted molecules (Liardon et al. 1991; Goodlett et al. 1995; Amelung and Brodowski 2002). We exploited this effect for the in vitro determination of the hydrolysis-induced racemization (HIR) of amino acids in each of pooled bulk soil and clay samples from the three agro-ecosystems' savanna sites and their ≥90 years old arable field plots, respectively.

The estimation of HIR followed the outline of Amelung and Brodowski (2002). The method involved a parallel hydrolysis with hydrochloric and deuterochloric acid (6 M, 12 h, 105 °C). The amino acids were then processed as described above. D-amino acids formed during ^{2}HCl hydrolysis incorporated deuterium into their Cα position. This gain in one atomic mass unit resulted in a relative signal loss of the non-deuterated fragment relative to the ^{1}HCl hydrolysate. The higher this signal loss, the higher is the HIR in a respective sample. Side-chain incorporations of deuterium were no limitation to this method as they could be estimated from that of the respective L-enantiomers. The concentration of D-amino acids formed upon hydrolysis were thus estimated by:

$$C_{\mathrm{D,rac}} = C_{\mathrm{D,}^{1}\mathrm{HCl}} - C_{\mathrm{D,}^{2}\mathrm{HCl}} - \Delta E, \tag{1}$$

with $C_{\mathrm{D,rac}}$ = concentration of the D-amino acid racemized upon hydrolysis, $C_{\mathrm{D,}^{1}\mathrm{HCl}}$ and $C_{\mathrm{D,}^{2}\mathrm{HCl}}$ = concentration of the D-amino acid after ^{1}HCl and ^{2}HCl hydrolysis, both quantified via the signal intensity of the non-deuterated mass fragment T_0, ΔE = error term.

The error term accounts for different D-amino acid yields between the two sample work-ups (Eq. (2)):

$$\Delta E = C_{\mathrm{D,}^{1}\mathrm{HCl}} - C^{\mathrm{total}}_{\mathrm{D}\ \mathrm{,}^{2}\mathrm{HCl}}, \tag{2}$$

with $C^{\mathrm{total}}_{\mathrm{D}\ \mathrm{,}^{2}\mathrm{HCl}}$ = total D-amino acid concentration in the ^{2}HCl hydrolysate.

This total D-amino acid concentration in the deuterochloric acid hydrolysate cannot be quantified via T_0, since it also comprises the deuterated fragments with the mass $T_0 + 1$u. Under accurate sample work-up, $C_{\mathrm{D}}^{\mathrm{total}}{}_{,^2\mathrm{HCl}}$ is the same as the measured D-amino acid concentration in the hydrochloric acid hydrolysate. In this case the error term approaches zero. If not, the error term may be estimated from the respective L-enantiomer concentrations, because the configuration of an amino acid does not affect its behavior during sample processing (see also Amelung and Zhang 2001):

$$\frac{C_{\mathrm{D}}^{\mathrm{total}}{}_{,^2\mathrm{HCl}}}{C_{\mathrm{L},^2\mathrm{HCl}}} = \frac{C_{\mathrm{D},^1\mathrm{HCl}}}{C_{\mathrm{L},^1\mathrm{HCl}}}, \tag{3}$$

with $C_{\mathrm{L},^1\mathrm{HCl}}$ and $C_{\mathrm{L},^2\mathrm{HCl}}$ = concentration of the L-enantiomer in the ^{1}HCl and ^{2}HCl hydrolysate as quantified via the T_0 target ion.

Resolving Equation (3) for the unknown $C_{D}^{\mathrm{total}}{}_{,^2\mathrm{HCl}}$, substituting this in Equation (2) and then into Equation (1) yields a final, accurate estimate of the D-amino acid concentration generated upon hydrolysis:

$$C_{\mathrm{D,rac}} = C_{\mathrm{D},^1\mathrm{HCl}}\left(\frac{C_{\mathrm{L},^2\mathrm{HCl}}}{C_{\mathrm{L},^1\mathrm{HCl}}}\right) - C_{\mathrm{D},^2\mathrm{HCl}}. \tag{4}$$

According to Equation (4), $C_{\mathrm{D,rac}}$ reflects the results of the ^{2}HCl hydrolysate. The proportion of D-amino acids formed during hydrolysis thus has to be referred to the total D-amino acid concentration in the ^{2}HCl rather than to that of the ^{1}HCl hydrolysate, yielding $100(C_{\mathrm{D,rac}}/C_{\mathrm{D}}^{\mathrm{total}}{}_{,^2\mathrm{HCl}})$ $(= C_{\mathrm{D},^1\mathrm{HCl}} - \Delta E$; Eq. (2)). Because of $C_{\mathrm{D}}^{\mathrm{total}}{}_{,^2\mathrm{HCl}} = C_{\mathrm{D},^1\mathrm{HCl}}$ multiplied with the ratio $\left(C_{\mathrm{L},^2\mathrm{HCl}}/C_{\mathrm{L},^1\mathrm{HCl}}\right)$ (Eq. (3)), we finally obtain for the HIR (Amelung and Brodowski 2002):

$$\mathrm{HIR}(\%) = 100\left(\frac{C_{\mathrm{D,rac}}}{C_{\mathrm{D}}^{\mathrm{total}}{}_{,^2\mathrm{HCl}}}\right) = 100\left[1 - \left(\frac{C_{\mathrm{D},^2\mathrm{HCl}}}{C_{\mathrm{D},^1\mathrm{HCl}}}\right)\left(\frac{C_{\mathrm{L},^1\mathrm{HCl}}}{C_{\mathrm{L},^2\mathrm{HCl}}}\right)\right]. \tag{5}$$

All HIR analyses were made in duplicate.

Decay model

Different rates of SOM decomposition have frequently been attributed to discrete pools of different stability (Jenkinson and Rayner 1977; Parton et al. 1987). For the South African sites, data on bulk C and N (Lobe et al. 2001) as well as on amino sugars (Amelung et al. 2002) supported the existence of at least two pools that could be estimated using a bi-exponential expression:

$$X_t = X_{\mathrm{l}} \quad 1em \quad \exp(-k_{\mathrm{l}}t) + X_{\mathrm{S}} \quad \exp(-k_{\mathrm{S}}t) \tag{6}$$

where X_t is the amino acid concentration at cultivation time t, X_{l} is the amino acid concentration of a labile pool, X_{S} is the amino acid concentration of a stable pool, and $X_{\mathrm{S}} = X_0 - X_{\mathrm{l}}$, where X_0 is the initial amino acid concentration in the grassland

($t = 0$), k_1 is the rate constant of the labile pool (year^{-1}), and k_S is the rate constant of the stable pool (year^{-1}). We did not use a three-pool model, as this is over-parametrized and does not significantly improve our data description.

To reduce data variability between sites, the amino acid concentrations (in mg kg^{-1} soil) were expressed relative to those of the grassland (i.e., $X_0 = 1$), which were assumed to be close to those of the original grassland site prior to land conversion for cropping. Due to the restricted data set it was not possible to use a bi-exponential model with an equilibrium amino acid concentration, as this resulted in an over-parameterization.

Statistical analyses

The decline in amino acid concentrations as well as changes in enrichment factors and amino acid D/L-ratios, were described by a bi-exponential model (Eq. (6)) using SigmaPlot for Windows 4.0 (SPSS Inc., Chicago, USA; automatic determination of initial parameters, 100 iterations, step size 0.1, and a tolerance of 0.000001). For the curve fitting, we used the Marquardt–Levenberg algorithm. Kinetic parameters of the models were calculated from the mean amino acid and N concentrations in the fractions of the three agro-ecosystems.

Data reduction

Amino acids comprise different structural elements. There are aliphatic amino acids without additional functional groups (e.g., alanine), amino acids with a second carboxylic (e.g., glutamic acid) or with a hydroxylic group (e.g., phenylalanine), basic amino acids with an additional amino group (e.g., lysine), sulfur-containing amino acids (e.g., methionine), and cyclic (e.g., proline) or hetero-aromatic amino acids. As all these amino acids have different chemical and ecological functions (Carey and Sundberg 1995), it seemed possible that their fate in soils is different. To reduce data size, we grouped the amino acids into basic (B-AA: lysine, ornithine), acidic (A-AA: glutamic acid, aspartic acid), and neutral amino acids as recommended by Christensen and Bech-Andersen (1989), but subdivided the neutral amino acids into two groups with either less than five carbon atoms (Ne1-AA: glycine, alanine, serine, threonine) or more (Ne2-AA: valine, leucine, isoleucine, proline, phenylalanine, methionine, tyrosine).

Results and discussion

Amino acids in the bulk soil

Amino acid-N identified about 30% of the total N content in the native and about 20% of N in the long-term cropped South African Plinthosols. The most common

amino acids in both bulk soil and clay fractions were glycine, alanine, serine, threonine and lysine, as well as the two acid amino acids glutamic and aspartic acid (Table 2 and 3). Each of the neutral amino acid classes comprised 30% of the total amino acids in the bulk soil. Additional 25% were acidic amino acids, and the basic ones added 15% to the total amino acid amount. A similar composition was reported by Christensen and Bech-Andersen (1989), reflecting that amino acid proportions in soil proteins may not be highly variable in different soils (Stevenson 1982), even not under different climates (Sowden et al. 1977).

Upon continuous arable cropping, amino acid concentrations decreased relative to both the soil organic C and N content in bulk soil and clay fractions (Table 4). The relatively large standard errors reflect the considerable variation of amino acid contents among the three agro-ecosystems (up to a factor of 4; Table 2 and 3). The result suggests that amino acids were preferentially used as substrates with increasing SOM decomposition as cropping period increased. This agrees with Gonzaléz-Prieto et al. (1997) who attributed amino acids to be a labile N pool which is easily mineralized. In addition, plant roots may utilize soil organic N in the form of amino acids (Jones 1999).

Describing the amino acid loss with a mono-exponential model (Du Toit et al. 1994) failed to predict the continuous decline in amino acid stocks at prolonged land use (Figure 2, dotted line). We therefore used the two-pool model approach (Eq. (6)). It explained 97% of our data variability (Figure 2). We conclude that, similar to amino sugars (Amelung et al. 2002), a labile and a stable pool can be distinguished also for amino acids in soil. The proportion of the labile pool in the savanna sites ($X_l = 62\%$ of the total amino acid amount) exceeded that of total N by a factor of 1.4. This explained the more rapid initial loss of amino acids relative to total N (Figure 2) or amino sugar-N (Amelung et al. 2002). In the long term, higher amino acid loss rates from the stable pool accounted for the progressive decline of amino acid-N proportions as arable cropping continued (Figure 2). The time of kinetic change, at which amino acid losses from the stable pool started to control dissipation kinetics (first derivative of $X_l \exp\{-k_l t\}$ is equal to that of $X_S \exp\{-k_S t\}$), was reached after 19.8 years. This demonstrates that soil organic N quality is significantly altered about two decades after breaking the savanna soil for cropping. The annual inorganic N fertilization could not compensate for this change.

The composition of the amino acids was only slightly altered by cropping period. Basic amino acids exhibited a rapid initial decline after grassland clearing; thereafter, the proportions of basic amino acids remained fairly constant (Figure 3). As a result, soil proteins tended to be depleted in basic amino acids in the short term but enriched in basic amino acids in the long term. This is also reflected by the elevated ratios of aspartic acid to lysine at the $<$8.5-year-old fields and the lowered ratios at the $>$90-year-old fields compared with those of the other fields (Table 4).

The loss rate constants were similar for the amino acids within each of the amino acid classes, except for the basic amino acids where ornithine was less rapidly lost than lysine (Table 2 and 3). This exception was probably due to different biochemical roles of these two basic amino acids. In contrast to lysine, ornithine is

Table 2. L-Amino acid concentrations[a] in fine earth (<2 mm) of the surface soils (0–20 cm) of the three agro-ecosystems Harrismith (HS), Kroonstad (KR), and Tweespruit (TW). Measured D/L-ratios ratios (in percentage of the respective L-enantiomer) are given in parentheses.

Cultivation period (years)	mg kg^{-1} soil															
	Gly	Ala	Ser[b]	Thr	Val	Leu	Ile	Pro[c]	Phe[b]	Met[b]	Tyr[b]	Lys	Orn[b]	Glu	Asp	Sum
Harrismith																
0	350	363 (13.3)	258	313 (0.4)	271 (1.9)	239 (1.7)	183 (0.0)	191 (1)	145	26	13	211 (6.8)	40	423 (16.5)	361 (13.3)	3387
3.5	199	192 (13.3)	155	172 (0.1)	146 (2.2)	116 (1.7)	96 (0.0)	88 (3)	75	11	6	119 (5.1)	26	245 (15.9)	211 (14.8)	1860
8	282	254 (13.5)	225	221 (0.4)	184 (1.8)	155 (2.0)	121 (0.4)	129 (1)	98	17	6	135 (4.9)	35	312 (16.3)	260 (14.2)	2434
10	174	171 (15.0)	149	151 (0.3)	132 (1.8)	111 (1.6)	85 (0.4)	85 (0)	65	12	6	102 (6.9)	26	223 (16.9)	178 (13.9)	1669
20	119	106 (15.3)	103	104 (0.5)	93 (2.1)	74 (1.7)	68 (1.4)	56 (0)	43	5	6	71 (5.7)	20	128 (17.0)	103 (15.1)	1100
30	96	91 (14.9)	84	83 (0.5)	73 (1.8)	62 (1.4)	56 (0.3)	48 (0)	40	5	3	64 (5.0)	18	137 (17.7)	104 (14.3)	963
45	115	125 (15.5)	75	105 (0.6)	108 (1.9)	85 (1.3)	69 (1.0)	65 (0)	59	8	6	83 (4.1)	20	175 (15.5)	121 (14.6)	1218
68	110	103 (12.8)	77	85 (0.6)	82 (1.7)	67 (1.3)	64 (0.6)	52 (0)	45	6	4	77 (4.3)	19	132 (16.8)	94 (14.2)	1017
90	115	98 (14.3)	85	82 (0.5)	76 (1.9)	64 (1.4)	58 (1.0)	45 (0)	36	4	8	66 (6.5)	19	102 (16.9)	81 (14.7)	938
Kroonstad																
0	148	99 (14.9)	132	146 (0.4)	98 (2.2)	114 (5.0)	93 (0.0)	108 (0)	78	21	33	130 (5.6)	37	256 (15.0)	184 (14.1)	1677
2.5	90	60 (16.0)	67	68 (0.6)	49 (2.6)	70 (3.8)	49 (0.0)	62 (3)	42	10	9	65 (7.7)	15	143 (16.6)	88 (13.2)	888
7.5	70	64 (18.2)	41	48 (0.0)	40 (2.8)	44 (4.0)	43 (0.0)	37 (4)	23	5	7	38 (5.2)	10	87 (18.2)	50 (14.0)	607
12	42	57 (16.9)	34	43 (0.7)	33 (2.2)	35 (4.0)	30 (0.0)	31 (5)	17	2	3	35 (8.1)	10	85 (21.8)	47 (15.8)	504
20	51	37 (14.7)	38	41 (0.0)	31 (1.9)	41 (4.1)	37 (0.0)	33 (3)	22	6	6	42 (5.3)	10	90 (18.1)	56 (12.9)	539
30	55	32 (16.7)	34	37 (0.0)	28 (1.7)	36 (4.3)	34 (0.0)	30 (4)	20	5	4	41 (5.4)	11	76 (18.5)	42 (13.2)	485
40	52	37 (14.3)	34	35 (0.4)	30 (1.4)	38 (3.1)	33 (0.0)	31 (3)	20	2	5	39 (5.5)	10	78 (16.3)	41 (12.6)	488
57	46	41 (32.4)	30	27 (4.9)	22 (0.8)	25 (2.8)	26 (3.5)	21 (4)	12	5	2	23 (5.0)	7	57 (17.2)	35 (11.1)	380
98	42	33 (23.0)	24	31 (1.1)	28 (2.1)	29 (3.0)	24 (0.0)	23 (4)	18	5	3	35 (8.7)	14	52 (17.7)	26 (12.3)	386

Table 2. (continued)

Cultivation period (years)	mg kg^{-1} soil															
	Gly	Ala	Ser[b]	Thr	Val	Leu	Ile	Pro[c]	Phe[b]	Met[b]	Tyr[b]	Lys	Orn[b]	Glu	Asp	Sum
Tweespruit																
0	190	132 (13.4)	222	212 (0.0)	138 (2.3)	169 (4.7)	135 (0.1)	147 (0)	115	16	12	188 (5.7)	59	369 (13.3)	274 (13.4)	2379
2	232	195 (15.2)	157	156 (0.0)	124 (1.7)	145 (3.7)	93 (0.0)	124 (3)	87	21	20	113 (4.4)	21	273 (13.1)	203 (10.7)	1964
8.5	137	102 (15.3)	123	110 (0.0)	80 (1.3)	91 (2.6)	67 (0.0)	68 (4)	62	16	25	90 (4.4)	26	229 (15.0)	166 (10.9)	1391
12	125	109 (14.9)	82	76 (0.7)	66 (2.2)	81 (3.5)	59 (0.0)	59 (4)	40	4	9	61 (4.4)	14	150 (14.3)	100 (11.4)	1035
22	98	77 (15.8)	63	65 (0.3)	53 (2.4)	60 (3.4)	49 (0.0)	47 (4)	32	7	5	52 (6.1)	14	117 (15.5)	78 (11.6)	815
32	88	66 (15.9)	48	56 (0.4)	48 (2.5)	49 (3.7)	59 (0.0)	38 (5)	26	6	3	49 (5.2)	13	112 (18.1)	63 (13.6)	725
40	74	58 (16.2)	43	47 (0.7)	42 (2.3)	44 (3.7)	38 (0.0)	32 (5)	24	3	4	38 (11.2)	11	85 (15.3)	51 (11.8)	595
60	88	65 (14.5)	51	59 (0.4)	43 (1.4)	50 (3.3)	50 (0.0)	42 (4)	28	9	5	47 (5.4)	13	114 (15.8)	65 (12.5)	729
90	51	46 (17.1)	35	51 (0.0)	44 (2.1)	41 (3.2)	42 (2.2)	31 (4)	24	10	4	47 (16.2)	16	81 (16.9)	40 (14.0)	563

[a] gly = glycine, ala = alanine, ser = serine, thr = threonine, val = valine, leu = leucine, ile = isoleucine, pro = proline, phe = phenylalanine, met = methionine, tyr = tyrosine, lys = lysine, orn = ornithine, glu = glutamic acid, asp = aspartic acid.

[b] D-forms not quantified.

[c] D-content almost exlusively due to hydrolysis-induced racemization.

Table 3. L-Amino acid concentrations[a] in the clay fraction (<2 µm) of the surface soils (0–20 cm) of the three agro-ecosystems Harrismith (HS), Kroonstad (KR), and Tweespruit (TW). Measured D/L-ratios (in percentage of the respective L-enantiomer) are given in parentheses.

Cultivation period (years)	mg kg^{-1} clay															
	Gly	Ala	Ser[b]	Thr[b]	Val	Leu	Ile	Pro[c]	Phe[b]	Met[b]	Tyr[b]	Lys	Orn[b]	Glu	Asp	Sum
Harrismith																
0	1012	716 (7.2)	1079	1071	678 (2.0)	758 (3.4)	666 (0.0)	629 (0)	533	1322	49	887 (4.0)	297	1766 (15.9)	1322 (13.6)	12790
3.5	861	753 (19.8)	617	651	626 (2.2)	553 (3.6)	532 (2.2)	345 (6)	306	61	99	403 (5.9)	125	927 (16.1)	747 (13.6)	7610
8	967	930 (15.7)	693	796	641 (2.0)	618 (2.5)	477 (0.0)	436 (5)	361	89	112	491 (4.9)	123	1287 (15.1)	992 (10.9)	9010
10	759	568 (14.0)	457	559	452 (2.6)	500 (3.5)	372 (0.0)	400 (3)	279	68	76	389 (4.3)	84	1006 (14.2)	738 (11.4)	6710
20	760	567 (16.3)	670	640	486 (1.9)	533 (3.0)	459 (0.0)	420 (5)	358	103	126	569 (5.2)	172	1339 (15.8)	950 (11.1)	8150
30	737	588 (19.3)	534	498	458 (1.9)	427 (3.4)	479 (0.0)	297 (6)	238	65	92	390 (3.7)	105	881 (17.9)	664 (13.0)	6450
45	664	586 (17.6)	488	461	391 (1.6)	393 (2.8)	346 (0.0)	282 (6)	221	65	75	344 (4.7)	93	920 (15.3)	645 (11.4)	5970
68	504	461 (19.0)	327	342	302 (2.0)	306 (2.3)	323 (0.0)	209 (6)	167	51	64	280 (4.5)	81	705 (17.1)	491 (11.5)	4610
90	508	457 (19.5)	321	387	303 (1.2)	350 (2.6)	276 (0.0)	248 (4)	188	69	73	299 (5.2)	90	782 (16.8)	510 (12.1)	4860
Kroonstad																
0	1157	1027 (18.0)	815	1014	705 (2.2)	579 (4.8)	611 (0.0)	534 (0)	313	1226	55	946 (5.4)	195	1506 (17.3)	1226 (14.3)	11910
2.5	674	565 (15.9)	447	511	401 (2.1)	412 (3.7)	361 (0.0)	330 (4)	225	63	59	359 (5.5)	80	858 (16.9)	611 (11.7)	5960
7.5	444	368 (19.3)	269	324	269 (2.5)	257 (3.4)	294 (1.3)	199 (5)	137	18	26	244 (5.1)	67	539 (18.8)	320 (12.8)	3770
12	437	390 (19.5)	252	313	250 (2.8)	235 (4.1)	295 (0.0)	199 (7)	124	23	19	237 (6.9)	69	606 (20.9)	378 (14.5)	3830
20	426	304 (17.3)	219	258	221 (2.3)	261 (3.3)	281 (0.0)	198 (5)	138	28	52	275 (5.8)	71	603 (19.2)	365 (12.9)	3700
30	397	265 (17.6)	206	230	187 (1.4)	236 (4.0)	277 (0.0)	197 (6)	129	14	14	265 (6.4)	79	597 (19.0)	353 (13.0)	3440
40	381	269 (14.3)	237	222	201 (1.9)	244 (2.8)	287 (0.0)	195 (4)	132	25	16	281 (6.1)	78	544 (19.6)	295 (13.4)	3410
57	337	234 (16.4)	238	211	174 (1.6)	208 (2.9)	229 (0.0)	175 (5)	114	30	22	241 (5.8)	72	534 (18.7)	317 (12.3)	3140
98	257	298 (21.2)	231	225	203 (2.1)	202 (2.9)	228 (2.5)	160 (4)	107	44	30	307 (20)	110	465 (19.4)	244 (12.5)	3110

Table 3. (continued)

Cultivation period (years)	mg kg^{-1} soil															
	Gly	Ala	Ser[b]	Thr[b]	Val	Leu	Ile	Pro[c]	Phe[b]	Met[b]	Tyr[b]	Lys	Orn[b]	Glu	Asp	Sum
Tweespruit																
0	1086	1177 (14.3)	718	955	899 (2.3)	651 (4.8)	660 (2.5)	482 (0)	339	832	10	593 (5.1)	112	937 (17.5)	832 (15.1)	10280
2	1150	821 (13.8)	958	881	619 (1.8)	828 (3.3)	550 (0.0)	657 (4)	581	105	124	800 (6.7)	160	1862 (14.5)	1435 (10.4)	11530
8.5	1053	906 (15.8)	729	822	677 (2.5)	625 (3.6)	547 (1.7)	445 (5)	364	62	83	519 (5.2)	105	1315 (16.2)	948 (11.5)	9200
12	598	537 (16.0)	410	414	372 (2.2)	385 (3.5)	348 (0.0)	253 (5)	210	9	19	306 (4.6)	82	730 (17.9)	502 (11.6)	5170
22	447	387 (20.5)	332	348	359 (2.0)	321 (3.5)	338 (0.0)	195 (6)	178	37	18	176 (3.9)	44	478 (18.8)	352 (13.2)	4010
32	446	382 (17.7)	259	251	235 (1.9)	229 (3.1)	345 (0.0)	169 (5)	124	22	8	236 (5.7)	69	573 (19.5)	344 (14.2)	3690
40	560	476 (14.7)	363	367	338 (2.5)	304 (3.2)	333 (0.0)	210 (5)	171	21	21	283 (5.1)	76	624 (17.2)	443 (12.3)	4590
60	453	330 (18.2)	287	304	274 (2.3)	245 (3.8)	343 (0.0)	189 (6)	138	44	50	254 (5.8)	77	531 (19.4)	340 (14.6)	3860
90	329	263 (18.9)	209	288	243 (2.7)	219 (3.1)	234 (3.0)	170 (4)	127	63	47	259 (10)	106	462 (18.8)	236 (14.1)	3260

[a] gly = glycine, ala = alanine, ser = serine, thr = threonine, val = valine, leu = leucine, ile = isoleucine, pro = proline, phe = phenylalanine, met = methionine, tyr = tyrosine, lys = lysine, orn = ornithine, glu = glutamic acid, asp = aspartic acid.

[b] D-forms below detection limit or not quantified.

[c] D-content almost exlusively due to hydrolysis-induced racemization.

Table 4. Organic C, total N, and amino acid concentrations in the fine earth (<2 mm) and clay fraction (<2 µm) of South African Plinthosols (0–20 cm) as affected by cropping period, shown as means of the three agro-ecosystems Harrismith, Kroonstad, and Tweespruit (standard errors are given in parentheses).

Length of cultivation (years)	Bulk soil (<2 mm)						Clay fractions (<0.2 µm)				
	pH[a] (H_2O)	C[b] ($g\,kg^{-1}$ soil)	N[b] ($g\,kg^{-1}$ soil)	Amino acids[c]		Asp/Lys[d]	C[a] ($g\,kg^{-1}$ clay)	N[b] ($g\,kg^{-1}$ clay)	Amino acids[c]		Asp/Lys[d]
				($g\,kg^{-1}$ C)	($g\,kg^{-1}$ N)				($g\,kg^{-1}$ clay-C)	($g\,kg^{-1}$ clay-N)	
0	5.5–6.0	13.4 (3.7)	1.19 (0.22)	196 (39)	2200 (443)	1.6 (0.1)	47.1 (3.7)	4.6 (0.2)	261 (16)	2670 (164)	1.5 (0.1)
2–3.5	5.0–6.3	9.2 (2.0)	0.93 (0.16)	181 (39)	1790 (385)	1.8 (0.2)	41.2 (6.1)	4.2 (0.5)	215 (42)	2110 (413)	1.9 (0.1)
7.5–8.5	5.1–5.6	8.3 (2.7)	0.79 (0.17)	189 (67)	1980 (706)	1.8 (0.2)	38.2 (8.3)	3.8 (0.6)	204 (49)	2050 (494)	1.8 (0.2)
10–12	4.8–5.7	7.0 (1.9)	0.70 (0.13)	162 (51)	1630 (506)	1.7 (0.1)	29.6 (4.8)	3.1 (0.3)	188 (29)	1800 (275)	1.8 (0.1)
20–22	5.4–5.8	5.9 (1.1)	0.60 (0.07)	147 (29)	1450 (282)	1.5 (0.0)	28.0 (4.4)	3.0 (0.3)	201 (54)	1880 (505)	1.8 (0.2)
30–32	5.2–6.1	5.8 (1.9)	0.65 (0.12)	133 (25)	1190 (226)	1.4 (0.2)	25.2 (5.0)	2.8 (0.3)	192 (40)	1730 (364)	1.6 (0.1)
40–45	5.7–5.9	5.5 (1.6)	0.59 (0.10)	148 (44)	1380 (412)	1.4 (0.1)	25.9 (3.8)	2.9 (0.2)	191 (30)	1710 (271)	1.6 (0.3)
57–68	5.7–6.5	5.0 (1.1)	0.56 (0.07)	151 (38)	1340 (342)	1.5 (0.1)	22.3 (2.8)	2.5 (0.2)	185 (20)	1650 (181)	1.6 (0.1)
90–98	5.2–6.1	4.4 (1.1)	0.53 (0.07)	152 (39)	1270 (290)	1.0 (0.2)	21.8 (3.5)	2.5 (0.3)	184 (27)	1610 (235)	1.2 (0.3)

[a]The pH values in 1 M KCl were, on the average, 1.2 units lower (see also Table 1).
[b]Data from Lobe et al. (2001).
[c]Calculated as the sum of glycine and 25 individual amino acid enantiomers.
[d]Asp/Lys is the ratio of aspartic acid to lysine.

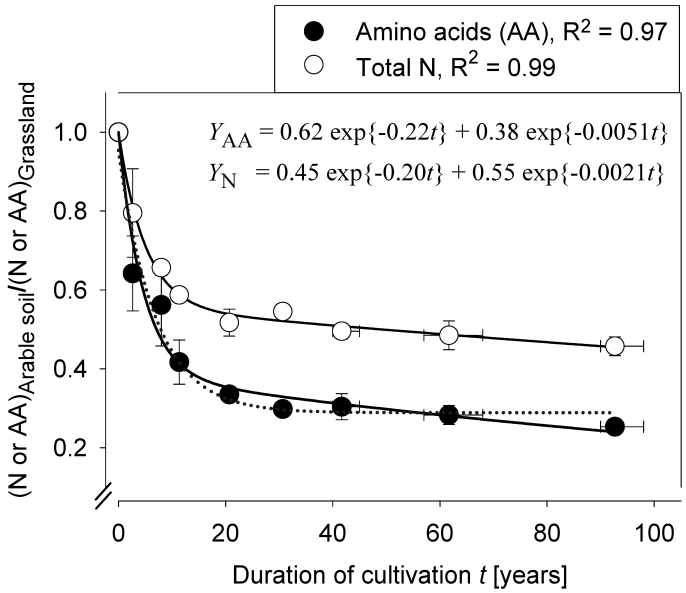

Figure 2. Total N and soil amino acid (AA) loss in the bulk soil (<2 mm) as affected by cultivation period, shown as means of the agro-ecosystems Harrismith, Kroonstad, and Tweespruit. Horizontal bars represent the span of cultivation period among the three agro-ecosystems, vertical bars the standard error.

usually not a protein-forming amino acid (Falbe and Regitz 1992; Szajdak and Österberg 1996). When considering the different amino acid classes, however, dissipation k_l rate constants increased in the order Ne1-AA < Ne2-AA = A-AA ≤ B-AA, but differences in k_l were small (Table 5). The relative pool sizes were similar for all amino acid classes. Hence, we cannot support the statement that turnover of proteins during arable land use is either associated with a preservation of basic amino acids (due to their higher inherent stability) or of acidic amino acids (due to their strong interaction with minerals; Stevenson 1982). Instead, Ne1-AA showed the lowest dissipation rates. As Ne1-AA comprised more than 50% glycine and alanine, commonly found in microorganisms, microbial re-synthesis of these amino acids probably compensated for losses in Ne1-AA.

Amino acids in the clay fractions

The SOM associated with the clay fractions is usually more stable against microbial decay than that of the coarser fractions and, hence, of the bulk soil (Buyanovski et al. 1994; Christensen 1996). We can confirm this for the hydrolyzable proteins. The decline in amino acid C and N concentrations was less pronounced for the clay fractions than for the bulk soil (Table 4). This slower dissipation was attributed to a

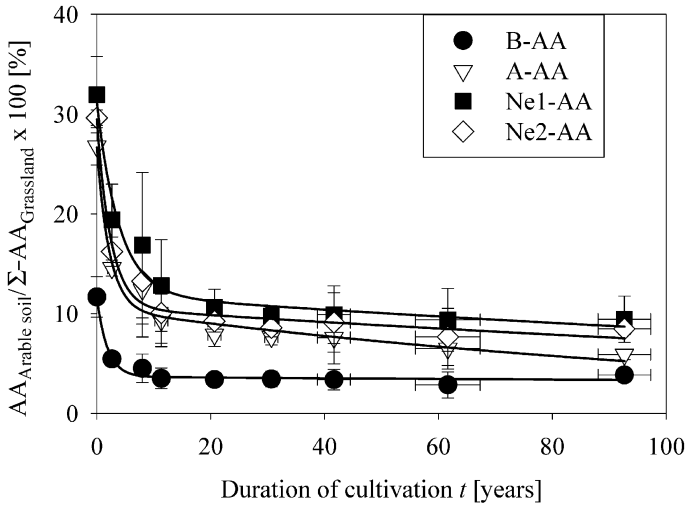

Figure 3. Loss of different amino acid classes in the bulk soil as affected by cultivation period, shown as means of the agro-ecosystems Harrismith, Kroonstad, and Tweespruit. Horizontal bars represent the span of cultivation period among the three agro-ecosystems, vertical bars the standard error (A-AA = Σ acid amino acids, B-AA = Σ basic amino acids, Ne1-AA = Σ neutral amino acids with <5 carbon-atoms, Ne2-AA = Σ neutral amino acids with ≥5 carbon atoms, Σ-AA = sum of all amino acids). Correlation coefficients are presented in Table 5.

smaller size of the labile X_l pool of the different amino acid classes, and significantly to lower dissipation rates of both acidic and basic amino acids from the clay than from other fractions (Table 5). Apparently, especially interactions of charged functional groups, such as of carboxylic groups with oxides (Kaiser et al. 1997) or of protonated amino groups with clay surfaces, contributed to a more effective preservation of amino acids in the clay fraction relative to the bulk soil.

In general, slower SOM losses from clay fractions may also indicate that material released during the decomposition of the coarser fractions shifted to the fine fractions (Zhang et al. 1988). In addition, microbial recycling of N in clay-sized microhabitats of the soil (Kandeler et al. 2000) can cause apparent slower turnover rates of clay-associated SOM. Nevertheless, as we analyzed mainly peptide-bound rather than free amino acids, such processes should affect not only the charged but also the neutral amino acids in the respective protein complexes. As this was not supported by our data, we conclude that a shift in amino acids among fractions and microbial amino acid recycling were less important for amino acid dynamics in the clay with prolonged arable cropping than direct interactions of the proteins with minerals.

Christensen (1992) characterized C pools by means of enrichment factors E that related element concentrations of a particular size fraction to the corresponding element concentration in the bulk soil (e.g., $E_{N,\ clay} = (g\,N\,kg^{-1}\,clay)/(g\,N\,kg^{-1}$ bulk soil)). The calculation of E values has the advantage that the SOM distribution

Table 5. Average kinetic parameters for the relative dissipation rates of amino acids in the bulk soil and the clay fraction at semi-arid grassland sites after cultivation in three agro-ecosystems in the Free State Province, South Africa (the standard errors created by parameter fitting are given in parentheses).

Class	Bulk soil[e]				Clay fraction[e]			
	X_l (%/100)	k_l (year^{-1})	k_S (year^{-1})	R^2	X_l (%/100)	k_l (year^{-1})	k_S (year^{-1})	R^2
Ne1-AA[a]	0.65 (0.06)	0.15 (0.03)	0.0027 (0.0031)	0.98	0.51 (0.08)	0.15 (0.05)	0.0044 (0.0031)	0.96
Ne2-AA[b]	0.62 (0.05)	0.24 (0.06)	0.0047 (0.0028)	0.97	0.56 (0.04)	0.31 (0.08)	0.0056 (0.0022)	0.97
A-AA[c]	0.57 (0.06)	0.28 (0.09)	0.0087 (0.0036)	0.97	0.52 (0.13)	0.09 (0.04)	0.0027 (0.0042)	0.95
B-AA[d]	0.62 (0.04)	0.45 (0.12)	0.0041 (0.0024)	0.97	0.61 (0.04)	0.19 (0.03)	0.0000 (0.0016)	0.98
Total	0.62 (0.06)	0.22 (0.06)	0.0051 (0.0032)	0.97	0.54 (0.06)	0.18 (0.04)	0.0040 (0.0023)	0.97

[a]Ne1-AA = glycine + alanine + serine + threonine.
[b]Ne2-AA = valine + leucine + isoleucine + proline + phenylalanine + methionine + tyrosine.
[c]A-AA = aspartic acid + glutamic acid.
[d]B-AA = lysine + ornithine.
[e]Parameters of the best model fit outlined in Equation (6).

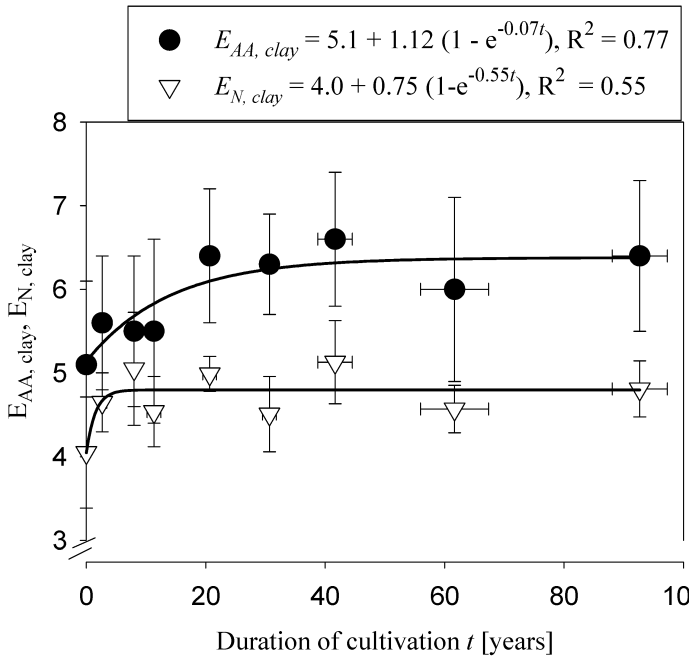

Figure 4. Effect of land-use duration on the average amino acid (AA) and N enrichment factors of the clay fraction, shown as means of the agro-ecosystems Harrismith, Kroonstad, and Tweespruit. Horizontal bars represent the span of cultivation period among the three agro-ecosystems, vertical bars the standard error.

among fractions can be compared irrespective of differences in SOM concentrations between sites. In the present study, this concept was also applied to the amino acid content ($E_{\mathrm{AA}} = (\mathrm{mg\ AA\ kg^{-1}\ clay})/(\mathrm{mg\ AA\ kg^{-1}\ bulk\ soil})$). Both $E_{\mathrm{N,\ clay}}$ and $E_{\mathrm{AA,\ clay}}$ exceeded unity, reflecting the higher total N and amino acid concentrations in the clay fractions than in the bulk soil (Table 4). As the loss rates of both clay-associated total N and amino acids were smaller than those in the bulk soil (see above), the E values increased exponentially to a maximum (Figure 4). This maximum was rapidly achieved for total N but slowly for amino acids, reflecting that differences between loss rates of the clay fraction and the bulk soil were less pronounced for amino acids than for total N. The high standard error between the agro-ecosystems, however, renders this enrichment factor only little suitable for characterizing protein dynamics in sites with different length of cropping.

Microbial effects on soil N dynamics

Decomposition and recycling of amino acid-N by the prevailing microbial community inevitably result in a synthesis of amino sugars. Amino sugars serve as

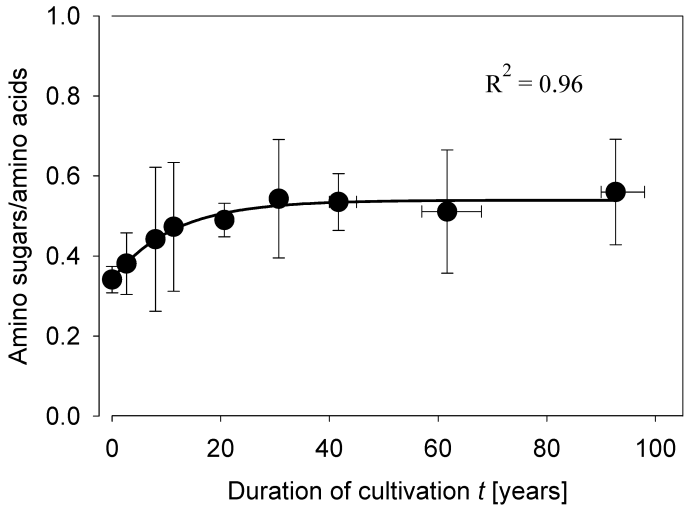

Figure 5. Ratio of amino sugars to amino acids in the bulk soil as affected by cultivation period, shown as means of the agro-ecosystems Harrismith, Kroonstad, and Tweespruit. Horizontal bars represent the span of cultivation period among the three agro-ecosystems, vertical bars the standard error.

markers for microbial residues in soil, because plants do not synthesize them on a significant scale (Stevenson 1982). To characterize the impact of microbes on soil organic N transformation, we calculated the ratio of amino sugars to amino acids. Previous work showed that the ratio increased with increasing SOM decomposition in incubation experiments or soil depth profiles (Amelung 2001). In this study, the ratio of amino sugars to amino acids was similar for bulk soil and clay fraction ($P>0.05$; paired t-test). It increased with increasing duration of cropping, but reached a maximum after about 20–25 years (Figure 5, only shown for the bulk soils; $R^2 = 0.72$ for the clay fractions). This time-span corresponded to the time of kinetic change for total N losses (22.6 years; Lobe et al. 2001). Yet, more research is required to accept or refute the hypothesis that a depletion of N from the labile pool terminates because the degree of microbial N transformation approaches steady-state equilibrium, especially since less than 30% of the N have been identified in the long-term fields. Moreover, we still know little about microbial transformations within the fractions of the hydrolyzable proteins. This takes us to the enantiomers.

Occurrence of D-amino acids in the Plinthustalfs

Several D-amino acids were detected in the samples, such as D-alanine, D-valine, D-threonine, D-proline, D-allo-isoleucine, D-leucine, D-aspartic acid, D-glutamic acid, and D-lysine. To ensure that they really occurred in the surface soil, it must be

Table 6. Hydrolysis-induced racemization of the amino acid enantiomers under study (standard error in parentheses).

Amino acid	% of total D-amino acid concentration found in soil			
	Bulk soil		Clay fraction (<2 μm)	
	Grassland	90 year cropping	Grassland	90 year cropping
D-Alanine	6.6 (1.5)	6.1 (0.1)	5.9 (1.7)	12.1 (3.1)
D-Valine	17.4 (2.1)	29.4 (3.7)	24.5 (0.3)	34.2 (0.6)
D-Threonine	n.d.	n.d.	n.d.	n.d.
D-Alloisoleucine	48.3 (0.2)	51.5 (2.0)	49.8 (0.4)	50.2 (0.5)
D-Isoleucine	0	0	0	0
D-Proline	73.8 (0.7)	71.3 (4.2)	80.7 (0.1)	71.1 (0.7)
D-Leucine	16.5 (1.4)	20.9 (0.9)	21.1 (0.7)	27.0 (0.1)
D-Aspartic Acid	28.1 (0.3)	29.8 (8.7)	33.2 (2.2)	24.6 (3.0)
D-Glutamic Acid	0	0	0	0
D-Lysine	34.3 (1.4)	23.8 (5.0)	27.9 (3.1)	28.4 (7.3)

n.d. = not determined (deuterium is re-exchanged during mass fragmentation).

excluded that they have been formed upon derivatization and hydrolysis from the respective L-enantiomers. Data on derivatization-induced racemization were reported by Amelung and Zhang (2001). Derivatization-induced racemization explained up to 50% of the D-valine, D-threonine, and D-isoleucine concentrations, up to 20% of the D-allo-isoleucine concentrations but less than 5% of those of the other enantiomers. To assess the amount of D-amino acids formed during hydrolysis, we used the deuterium labeling technique and calculated HIR according to Equation (5). The results revealed that about 50% of the D-allo-isoleucine and more than 70% of the D-proline concentrations must be attributed to HIR (Table 6). We conclude that these two D-amino acids only existed in soil at trace amounts and do not indicate protein ageing as previously suggested (Mahaney and Rutter 1989; Miller et al. 1997). For the other D-amino acids, HIR accounted for $\leq$30% of the detected contents, and little if any HIR was found for the bacterial biomarkers D-alanine and D-glutamic acid (Table 6). Except for D-valine, the HIR was also similar for all tested samples, irrespective of whether they comprised bulk soil or clay fraction or savanna or cropland. This suggested that there was little if any risk that changes in the D-amino acid concentration among fractions or in course of prolonged cropping were caused by changes in HIR. In the following, we therefore restricted the evaluation of the amino acid-enantiomer signature in soil to the D/L-configurations of alanine, leucine, aspartic acid, glutamic acid, and lysine.

Effect of cultivation period on the D/L-ratios in bulk soil and clay fraction

Except for glutamic acid, the D/L-ratios of amino acids in the clay fraction were similar to those assessed for the bulk soil ($P > 0.05$). This was because the clay

fractions contained about 75% of the total amino acids in soil. The D/L ratios of glutamic acid were slightly higher in the clay fractions than in the bulk soil ($P < 0.05$; Table 2 and 3). This D-glutamic acid originated from bacterial peptidoglycan (Voet and Voet 1995), which was preferentially enriched in the clay-associated SOM (Amelung et al. 2002). Compared with D-alanine, which also derived from bacterial cell wall (Voet and Voet 1995), the additional carboxylic group of glutamic acid may promote its sorption to oxides (Kaiser et al. 1997).

Due to the similarity of the amino acid-enantiomer signature in bulk soil and clay fractions, we restricted the discussion of cultivation effects to the clay fractions. Compared to the bulk soil, these exhibited a smaller analytical error, due to the lack of material that floats during hydrolysis. The D/L ratio of glutamic acid responded similarly to cultivation in both the bulk soil and clay fractions.

With increasing period of arable land use, the D/L ratios of individual amino acids changed, but into three different directions. (i) The D/L ratios increased for glutamic acid and alanine (Figure 6(a), only shown for alanine), but (ii) decreased for leucine and initially aspartic acid (Figure 6(b), only shown for aspartic acid). In contrast, (iii) the D/L ratios remained constant for lysine for the first 60 years of cropping but were significantly higher for the >90-year-old cultivated fields (Table 2 and 3).

(i) Both D-alanine and D-glutamic acid are constituents of the bacterial cell wall (Voet and Voet 1995). As the absolute concentrations of these two amino acids decreased upon cultivation (Figure 2), an increase in the proportion of the respective D-enantiomer merely implied that bacterial residues were less rapidly lost than other amino acids and the corresponding L-enantiomers. We attribute this finding to three reasons. First, bacteria, living in close proximity to clay surfaces, synthesize these amino acids with increasing SOM alteration as duration of cropping proceeded. Second, the bacterial peptidoglycan is likely to be discriminated against other proteins during soil N transformation, because the D-alanine protects it from bacterial proteases (Voet and Voet 1995). Third, soil microorganisms mineralize D-amino acids at a slower rate than the corresponding L-enantiomers (Hopkins and Ferguson 1994; O'Dowd et al. 1997), thus also promoting their relative accumulation.

    It should be noted that the ratio of D-alanine N to D-glutamic acid-N exceeded unity and averaged 1.3 in both bulk soil and clay fractions (standard error 0.08 and 0.09, respectively). Thus, there was slightly more N in D-alanine than in D-glutamic acid. The ratio of D-alanine N to muramic acid-N (data from Amelung et al. 2002) even slightly exceeded 2 (not related to cropping period, data not shown), despite bacterial peptidoglycan usually contains both compounds in similar amount. The results possibly suggested that there were additional D-alanine sources, such as teichoic acids, which could be clearly detected by ^{31}P nuclear magnetic resonance spectroscopy (Lobe unpublished data).

(ii) The D-content of aspartic acid was used to calculate the age of human enamel or dentin (Bada 1985). The D/L ratio of aspartic acid also correlated

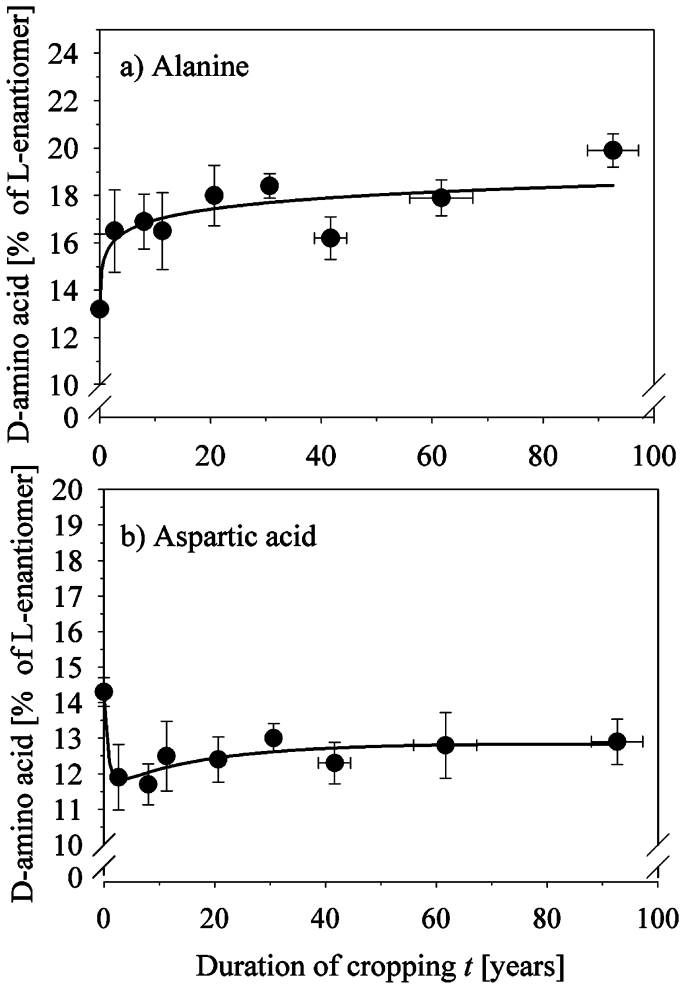

Figure 6. Changes in the D-amino acid proportions (percentage of the L-amino acid concentration) of alanine and aspartic acid of the clay fraction as affected by cultivation period, shown as means of the three agro-ecosystems Harrismith, Kroonstad, and Tweespruit. Horizontal bars represent the span of the cultivation period among the three agro-ecosystems, vertical bars the standard error.

closely with ^{14}C age in soil and sediments, reflecting different protein age in environment (Harada et al. 1996; Amelung 2001). The rapid decline of aspartic acid D/L ratios in the clay fractions upon cropping (Figure 6(b)), therefore, implied that aged soil proteins were decomposed and/or replaced by fresh plant-derived proteins which comprise little if any D-amino acid residues. Whether there was a tendency for a re-increase of the D/L ratio of aspartic acid in the long term (Figure 6(b)) is not statistically evident from the data. More research is also required to elucidate the respective

mechanisms. Nevertheless, breaking of grassland soil results in a rapid loss of macroaggregates (Elliott 1986; Gupta and Germida 1988), as it was also confirmed for the sites under study (Lobe unpublished data). Macroaggregates, however, physically protect plant-derived organic matter from decay (Beare et al. 1994; Amelung and Zech 1996). Hence it seems reasonable to speculate that old plant proteins became accessible to decay after the rapid breaking of aggregates. A rejuvenation of SOM after conversion of grassland into arable land was also suggested by the analysis of lignin-derived phenols, which indicated a replacement of oxidized lignin by fresh lignin residues as a result of aggregate breakdown and losses of old silt-associated lignins by wind erosion (Lobe et al. 2002).

The racemization rates of aspartic acid are likely mediated by microbial processes, as evident from the different ^{13}C natural abundance of its enantiomers (Glaser and Amelung 2002). With the current information available, it is, therefore, presently not possible to use the D-content of aspartic acid for an absolute dating of the soil proteins. Nevertheless, the opposite development of the D/L ratio of aspartic acid relative to the D/L ratio of alanine after cropping the soil gives support to the conclusion of Amelung (2001) that changes in the D-content of soil-borne aspartic acid does indicate really an ageing of a bioavailable soil protein fraction and not an accumulation of microbial cell remains.

Next to aspartic acid, also the D/L ratios of leucine decreased exponentially with increasing duration of cropping ($r^2 = 0.80$ for the bulk soil and 0.53 for the clay fractions Table 2 and 3). However, it is yet uncertain whether this also indicated a rejuvenation of proteins similar to the D/L ratio of aspartic acid, because there was no systematic change in the D/L ratio with increased organic matter age in fossil soils or radiocarbon-dated soil profiles (Amelung 2001).

(iii) Previous studies revealed that beside aspartic acid the D/L ratio of lysine was closely correlated with SOM age, irrespective of the degree of microbial N status (Amelung 2001). In the present samples, the D/L ratio of lysine remained unchanged, suggesting that cropping effects on total SOM age were only little if at all pronounced. Compared to aspartic acid, lysine racemization rates are slower (Frank et al. 1982), that is, they are a less sensitive tool to elucidate relative ageing of proteins upon cropping practice. Nevertheless, the D/L ratio of lysine is significantly higher in the 90-year-old fields compared to the other fields ($P < 0.05$, ANOVA). Possibly, the presence of additional D-lysine in these fields reflects an impact of early land-use practice on a part of SOM that could have been preserved during cropping. Alternatively, the higher D/L ratio of lysine might indicate that these fields contained more subsoil, that is, older SOM, due to continued losses of surface SOM by wind erosion (Lobe et al. 2001). Identifying the origin of D-lysine in these soils, for example, with the assistance of compound-specific natural isotope-abundance measurements, might thus warrant further investigations.

Conclusions

In this study the amino acid signature was assessed for coarse-textured South African Plinthustalfs that have been converted from grass- to cropland since a few years to almost one century ago. After 20–30 years of cropping already 70% of the initial amino acid content was irretrievably lost, even in preference to other soil N structures. Thus, hydrolyzable amino acids comprise a labile rather than a stable soil N pool. As this pool cannot be affected by the type of rotation (Senwo and Tabatabai 1998), it should be re-inserted by legume green manure better than by fallow practice (Campbell et al. 1991).

Different amino acid enantiomers responded differently to cropping duration. Thus, different amino acid enantiomers can be used to elucidate different processes of soil N dynamics. From our data, we inferred that intensified cropping of arable land in this region increased the relative proportions of microbial-derived N at the initial expense of old soil protein reserves.

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