

María-Belén Turrión · Bruno Glaser · Wolfgang Zech

Effects of deforestation on contents and distribution of amino sugars within particle-size fractions of mountain soils

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Abstract Identifying amino sugar pools in soil may advance our knowledge about microbially derived substances. Our objective was to study the effects of deforestation followed by pasture establishment on amino sugar contents and patterns in mountain soils of the Sui Checti Valley in the Alay Range, Kyrgyzia. Soil A horizons (0–10 cm) under pasture and forest were fractionated into clay, silt, fine and coarse sand fractions. Soil organic C, N, glucosamine (GlcN), galactosamine, mannosamine and muramic acid (MurAc) were analysed in the fine earth, particle-size fractions and humus layers. Deforestation of *Juniperus turkestanica* and pasture establishment led to a significant increase in the amino sugar content in the fine earth and soil organic matter (SOM) reflecting different accumulation of amino sugars in different ecosystems. Deforestation affected principally the amino sugars in the coarse and fine sand fractions that generally are related to particulate organic matter. High concentrations of amino sugars were found in the clay fraction, corroborating the general assumption that it contains a high microbial biomass and microbially altered organic matter. The pasture soil samples have a significantly higher GlcN to MurAc ratio indicating a higher fungal contribution to SOM than in forest soils. Although other studies revealed that pasture is associated with high amounts of bacterial amino sugars, our study gave evidence that deforestation tends to lead to the accumulation of mainly fungi-derived amino sugars. It is proposed that this might be due to a lowering of soil pH.

Keywords Land use · Deforestation · Mountain soils · Amino sugars · Particle-size fractionation

Introduction

Land use affects soil organic matter (SOM) dynamics (Guggenberger et al. 1994; Zhang et al. 1999). Deforestation followed by pasture establishment in mountain soils of the Alay Range, Kyrgyzia, led to a loss of about 30% of total organic C (TOC) and to an accumulation of about 20% of N and 50% of P compared to the native *Juniperus turkestanica* forest stands (Glaser et al. 2000; Turrión et al. 2000). Changes in soil management practices influence not only the amount but also affect the composition of SOM (Tiessen and Stewart 1983; Cambardella and Elliot 1992; Christensen 1992; Guggenberger et al. 1994; Glaser et al. 2000; Turrión et al. 2000). Many studies have been carried out to investigate the influence of land-use changes on tropical (Guggenberger et al. 1995; de Moraes et al. 1996), temperate (Tiessen and Stewart 1983; Guggenberger et al. 1994) and high mountain soils (Glaser et al. 2000; Turrión et al. 2000). However, the effects of deforestation followed by pasture establishment on microbially derived residues in soils are still unclear. Such information may help to deduce the microbial transformation of SOM under different land-use systems.

Physical fractionation of soil according to particle size combined with degradative chemical techniques was shown to be a potent tool for evaluating land-use-induced changes in SOM (Guggenberger et al. 1994; Glaser et al. 2000; Turrión et al. 2000). This fractionation is based on the concept that SOM fractions associated with particles of various size classes and mineralogical composition differ in structure and function and, therefore, play different roles in SOM turnover (Christensen 1992). Particulate organic matter is assumed to be associated with the sand fraction, SOM with medium-term turnover in the clay fraction, and SOM with long-term turnover in the silt fraction (Guggenberger et al. 1995; Zech and Guggenberger 1996). Guggenberger et al. (1994) and Glaser et al. (2000) utilized neutral sugar abundance and lignin composition in SOM of particle-size separates to evaluate the influence of different land-use practices on

M.-B. Turrión (✉)
Area of Soil Science and Soil Chemistry, E.T.S.I.A.,
University of Valladolid, 34004 Palencia, Spain
e-mail: bturrión@agro.uva.es

B. Glaser · W. Zech
Institute of Soil Science and Soil Geography,
University of Bayreuth, 95440 Bayreuth, Germany

SOM dynamics in tropical and high mountain soils, respectively. Although such parameters help to assess plant- and microbe-derived sources of SOM, they are not adequate to differentiate between compounds of different microbial origin (Zhang et al. 1999).

During SOM transformation, microbes build-up and decompose cell-wall constituents. In the short term, SOM transformations are thus linked to fluctuations in living and dead microbial biomass (Van Veen et al. 1984). Several methods can be used to assess living soil biota (Alef and Nannipieri 1995), but very few are suitable for the determination of microbial C and N residues in soil. Among them, analyses of amino sugars are helpful in the assessment of both living and dead biomass and their residues in soil (Amelung 2000). The amino sugar pattern can be used as an indicator of the origin of different microbial C and N residues in soil because these compounds are part of the cell walls of bacteria, fungi and actinomycetes. Bacterial cell walls contain a peptidoglycan, which is constructed from the glucose derivatives *N*-acetyl glucosamine and muramic acid (MurAc). The peptidoglycan structure is present only in prokaryotes, but has never been found in eukaryotic cells. Fungi contain only glucosamine (GlcN) and galactosamine (GalN) (Cochran and Vercellotti 1978). The origin of mannosamine (ManN) is controversial (Coelho et al. 1997). Zhang and Amelung (1996) used MurAc, GlcN, ManN and GalN to elucidate the microbial origin of soil organic N. There is evidence showing that the amino sugar recognized to be fungal in origin, GlcN, also exists in the chitin of arthropods as well as in higher plants as a constituent of glycoproteins (Zhang et al. 1999). However, for soil studies, Sowden and Ivarson (1974) found that little GalN was synthesized during a fungi-inoculated incubation. Chantigny et al. (1997) also successfully used MurAc and GlcN to differentiate between bacterial and fungal contributions to soil aggregation. Amino sugar analyses are suitable for the determination of microbial C and N residues in soil, whereas conventional methods of biomass estimation such as fumigation-extraction, substrate-induced respiration, or microbial-diversity indicators such as phospholipids or ergosterol are used to assess living soil biota which show big seasonal fluctuations (Amelung 2000). Amino sugar analyses, on the

other hand, have successfully been applied to the elucidation of land-use changes in temperate (Zhang et al. 1999) and tropical (Solomon et al. 2001) environments. However, nothing is known so far of how land-use changes influence the amino sugar pattern of mountain soils, which is the intention of this paper.

Materials and methods

Materials

The study was carried out in the Sui Checti Valley, which is situated in the Alay Range, Kyrgyzia at about 2,900 m a.s.l. (40°02'N, 72°39'E; Glaser et al. 2000; Turrión et al. 2000). At the research site, *J. turkestanica* forests ascend up to about 3,200 m a.s.l. These forest remnants are increasingly disturbed by grazing and uncontrolled logging. Cows and goats intensively graze the pasture soils in summer. The mean annual temperature is -0.1°C and mean annual precipitation is 900 mm (Pertziger 1996). The soils under study were classified as Borolls (Glaser et al. 2000). Generally, the soils are stratified with silty surface horizons and sandy loamy subsurface layers, with high stone contents, especially on steep slopes.

To analyse the effects of deforestation on SOM quantity and quality, we took representative samples of litter, humus layers and five replicates of soil samples from the mineral A horizon (0–10 cm) below the canopy of dense microstands of juniper and pasture soils in the vicinity of the forest stands for comparison according to a radial sampling scheme (Wilding 1985). General soil characteristics of humus layers and A horizons under forest and pasture are given in Table 1. More details are published elsewhere (Glaser et al. 2000; Turrión et al. 2000). Description and analyses were carried out according to Schlichting et al. (1995) and Soil Survey Staff (1997). No significant differences between the texture of the investigated soils could be observed, assuming that all investigated soils are comparable.

Methods

All analyses were carried out on air-dried soil samples sieved through a 2-mm screen (fine earth). For particle-size fractionation, a 30-g soil sample was dispersed ultrasonically in 150 cm³ water using a probe-type disintegrator (Branson Sonifier W450). The energy output of the sonifier was determined calorimetrically according to North (1976). Particle-size classes were separated according to Amelung et al. (1998). Coarse sand (2,000–250 µm) was separated after an energy input of 60 J cm⁻³ by wet sieving. After an additional energy input of 460 J cm⁻³, fine sand (250–20 µm) was separated by wet sieving, silt (20–2 µm) and clay (<2 µm) by sedimentation. Coarse sand and fine sand were

Table 1 General properties of humus and A horizons under forest and pasture of mountain soils of the Alay Range, Kyrgyzia. Yield of size fractions and the sum of the yield (g kg⁻¹). ND Not determined

Horizon	Thickness (cm)	pH (CaCl ₂)	Forest			Pasture		Sand (g kg ⁻¹)	Silt	Clay	Sum
			TOC (g kg ⁻¹)	N	C/N	TOC (Mg ha ⁻¹)	N				
Forest											
L	1.5	ND	465.7	11.5	41	10.5	0.3	–	–	–	–
Of	3	7.1	340.3	15.5	22	16.3	0.7	–	–	–	–
Oh	2	7.2	258.6	13.2	20	10.3	0.5	–	–	–	–
Ah	10	7.0	130.8	8.6	15	170.0	11.2	354	441	155	950
Pasture											
Ah	10	5.6	87.7	11.5	8	114.0	14.9	275	453	199	927

dried at 40°C, silt and clay were freeze dried prior to chemical analyses. Destruction and redistribution of labile particulate organic matter was minimized by using a two-step sonification procedure as suggested by Amelung and Zech (1999).

TOC and total N in soil samples and particle-size separates were determined by dry combustion on a Vario EL C/N analyser.

Amino sugars were determined according to Zhang and Amelung (1996). About 500 mg of sample aliquot was hydrolysed with 6 M HCl at 105°C for 8 h. The released amino sugars were separated from impurities by neutralization with KOH. Derivatization was carried out according to Guerrant and Moss (1984). The aldonitrile derivatives were analysed on an HP 6890 gas chromatograph equipped with an HP-5 fused silica column and a flame ionization detector. The amino sugars were quantified using inositol and *N*-methyl GlcN as internal standards.

Statistical analysis

All data were statistically analysed using Excel 97 or Statistica 5.1 for Windows NT 4.0. Normal distribution of the data were tested using the David test. The homogeneity of the variances of the normal distributed data was tested with an *F*-test. Significant differences between the mean measurements among forest and pasture were tested subsequently with an adequate *t*-test (Hartung et al. 1993; Sokal and Rohlf 1995). The non parametric *U*-test (Mann-Whitney) was applied for these samples unless the prerequisites for the *t*-test were met (Sokal and Rohlf 1995). Differences between size fractions within one land-use system were tested with MANOVA followed by a Scheffé-test.

Results and discussion

Amino sugars in the humus layer and in the fine earth

Table 2 shows the amino sugar concentrations in the forest humus layers and in the mineral A horizon of the soils under investigation. With increasing litter decomposition from the L to the Oh horizon, the concentrations of GlcN, GalN, MurAc and the total amino sugar concentrations referred to dry weight increased, GlcN being the most abundant amino sugar monomer followed by GalN. Referred to TOC, all amino sugar monomers increased with progressing litter decomposition indicating that the SOM is more and more enriched in microbially altered organic matter. The accumulation of thick forest floor layers below the canopy of junipers at our research

site reduced mineralization of the SOM components. High microbial biomass, however, correlates usually with high SOM mineralization (Hassink 1994). Therefore, the high amino sugar concentrations in the forest floor layers indicate a stabilization of amino sugars in the SOM rather than a high microbial biomass. This general difference between amino sugar and microbial biomass estimates was also suggested by Amelung (2000).

Compared with the forest soil, deforestation significantly ($P < 0.05$) increased the amino sugar content in the fine earth of the A horizon from 9.2 g kg⁻¹ to 11.4 g kg⁻¹ soil. The forest organic layers had the highest amino sugar concentrations (up to 20 g kg⁻¹). Although the humus layers contain very high amino sugar concentrations (Table 2) they contribute only a minor part (2.0 Mg ha⁻¹) to the total amounts of amino sugars of 10.2 and 15.3 Mg ha⁻¹ in forest and pasture soils, respectively. Previous studies in the same area showed that deforestation followed by pasture establishment led to a loss of about 30% of TOC (Glaser et al. 2000). The contribution of amino sugars to SOM (g amino sugar C kg⁻¹ TOC of fine earth) is more than twice as high in the mineral soil horizons of pasture as in forest soil (Table 2), which reflects a higher contribution of microbial residues to the SOM of the pasture soil. Srivastava and Singh (1991) reported that the conversion of dry tropical forest into other land-use systems resulted in a marked decline of the amounts of soil nutrients and microbial C, N and P. Zhang et al. (1999) compared amino sugar pools from three different land-use systems: native pasture, arable land, and a 75-year-old forest; all of these soils were Argiudolls. The authors observed that at both cultivated and forested sites the concentrations of amino sugars in SOM were lower than that at the pasture site.

The ratio of GlcN to MurAc reflects the impact of fungi and bacteria on the SOM (Zhang and Amelung 1996; Zhang et al. 1999; Amelung 2000; Solomon et al. 2001). Hence, GlcN/GalN and GlcN/MurAc ratios are useful for illustrating the origin of amino sugars from different microbes in most cases.

Pasture soil samples have a significantly higher GlcN/MurAc ratio indicating a higher fungal impact in

Table 2 Concentrations of glucosamine (*GlcN*), mannosamine (*ManN*), galactosamine (*GalN*), muramic acid (*MurAc*), total amino sugars (*Total AS*) and ratios of glucosamine to mannosamine

(*GlcN/ManN*), glucosamine to galactosamine (*GlcN/GalN*) and glucosamine to muramic acid (*GlcN/MurAc*) in humus layers and mineral A horizons. *DW* Dry weight, *TOC* total organic C

		GlcN ManN GalN MurAc Total AS					GlcN ManN GalN MurAc Total AS					GlcN/ ManN	GlcN/ GalN	GlcN/ MurAc	
		(g AS kg ⁻¹ DW)					(g AS C kg ⁻¹ TOC)								
Forest															
L	1.5 cm	5.6	1.4	0.7	2.0	9.7	4.8	1.42	0.6	1.8	8.5	4.1	7.7	2.8	
Of	3 cm	11.2	0.3	5.8	2.6	19.9	13.3	0.4	6.8	3.2	23.7	36.1	1.9	4.4	
Oh	2 cm	11.4	0.3	6.0	2.2	19.9	17.9	0.5	9.5	3.5	31.4	35.3	1.9	5.3	
A	10 cm	4.8	0.6	2.5	1.3	9.2	14.3	1.9	7.5	4.1	27.8	8.0	1.9	3.7	
Pasture															
A	10 cm	7.1*	0.3	3.5*	0.5	11.4*	30.4***	1.3	15.1**	2.1	48.9***	23.7*	2.0	14.2**	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (for significant differences between forest and pasture)

Table 3 Concentrations of GlcN, ManN, GalN, MurAc, Total AS and GlcN/ManN, Glc/Gal and GlcN/MurAc in particle-size fractions of A horizons of forest and pasture soils. *Different letters*

indicate significant differences ($P < 0.05$) between different size fractions within one land-use system. For abbreviations, see Table 2

	GlcN	ManN	GalN	MurAc	Total AS	GlcN/ManN	GlcN/GalN	GlcN/MurAc
	(g AS kg ⁻¹ DW)							
Forest								
Coarse sand	5.1±0.6a	0.7±0.3a	2.4±0.2a	1.2±0.2a	9.4±0.6a	7.3a	2.1a	4.2a
Fine sand	6.6±2.2a	2.7±0.9b	2.4±1.3a	2.6±0.3b	14.3±5.0a	2.4a	2.8a	2.5b
Silt	4.1±2.3a	0.8±0.6a	2.3±0.6a	3.6±0.5b	10.8±3.0a	5.1a	1.8a	1.1b
Clay	11.1±1.0b	0.3±0.1a	6.0±1.2b	4.9±0.4c	22.3±1.3b	37b	1.9a	2.3b
Pasture								
Coarse sand	6.2±2.7a	0.14±0.02a**	2.5±0.9a	0.3±0.1a**	9.2±2.3a	44.3a**	2.5a	20.7a**
Fine sand	7.1±4.1a	0.8±0.3ab**	3.2±2.1a	0.4±0.1a**	11.7±5.2a	8.9b**	2.2a	17.8a**
Silt	3.2±0.2a	1.6±0.5b	1.4±0.4a	3.1±1.5b	9.3±2.3a	2.0c	2.3a	1.0b
Clay	12.0±0.6b	0.4±0.1a	6.9±0.8b	4.3±1.0b	23.6±1.8b	30a	1.7a	2.8b

** $P < 0.01$ (for significant differences between forest and pasture)

pasture compared to forest soils (Table 3), probably due to the lower pH values in pasture soils (Table 1). Lower pH values normally favour fungi. Zhang et al. (1999) concluded in their study about land-use effects on amino sugars of an Argiudoll that the pasture favours the accumulation of bacterial amino sugars, whereas forestation favours accumulation of the mainly fungi-derived amino sugar GlcN. Our study, however, showed opposite results. From these results it might be concluded that instead of the land-use system (pasture), the soil pH is the variable dominating the predominance of fungi or bacteria, although other factors might also be responsible for this phenomenon.

Amino sugars in particle-size fractions

The clay fraction showed the highest amounts of GlcN, GalN, MurAc and total amino sugars, both in forest and pasture soils (Table 3), probably due to stabilization of microbial metabolites by association with clay minerals protecting the amino sugars from mineralization. On the other hand, it is well known that clay fractions contain high amounts of microbial biomass and microbial activity (Ladd et al. 1996). Therefore, high amino sugar contents in this fraction might predominately result from the biomass itself (living and dead compounds) which might represent the main part of labile C within clay-associated SOM. These results indicate that organic matter in the clay fraction still comprises labile SOM pools, despite the fact that this fraction is considered to contain stabilized SOM (Christensen 1992).

The ratio of GlcN to MurAc decreased with decreasing particle size both in forest and pasture soils, indicating an increasing impact of bacterial degradation with decreasing particle size. The significantly ($P < 0.05$) higher GlcN concentrations in the clay fraction compared to other particle-size fractions, however, gives evidence that fungal degradation products still play an important role in clay-sized particles. It cannot be excluded, however, that amino sugars, especially in the clay

fraction, are derived at least partly from living biomass. The higher ratios of GlcN/ManN and GlcN/MurAc in the sand and clay fractions of pasture compared to those in the forest soil corroborate the results obtained for fine earth, suggesting that higher soil pH favours a microbial population dominated by bacteria, whereas the lower pH of the pasture soil favours the accumulation of mainly fungi-derived amino sugars. Zhang et al. (1999) showed that pasture favours the accumulation of bacterial amino sugars and cultivation and forestation practices favour accumulation of fungal amino sugars, but the studied soils presented similar soil pH values.

In conclusion, in the Sui Checti Valley of the Alay Range, Kyrgyzia, deforestation of *J. turkestanica* followed by pasture establishment led to a significant increase in amino sugar concentrations in the soil surface (0–10 cm). Deforestation caused an enrichment of amino sugars in the SOM reflecting the different quality of SOM between these two land-use systems. Also, the concentration of amino sugars in particle-size fractions was influenced by deforestation affecting principally amino sugars related to coarser size fractions which are generally related to particulate organic matter. High concentrations of GlcN, GalN, MurAc and total amino sugars in the clay fraction revealed that SOM in the clay fraction comprises labile SOM pools, despite the fact that this fraction is considered to contain relatively stabilized SOM. It cannot be excluded that living biomass significantly contributed to this labile SOM. Pasture soil samples have a significantly higher GlcN/MurAc ratio indicating a higher fungal contribution compared to forest soils.

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