Comparison of organic compounds in the particle-size fractions of earthworm casts and surrounding soil in humid Laos

Xudong Zhang a,∗ Jing Wang a, Hongtu Xie a, Jingkuan Wang b, Wolfgang Zech c

a Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, CAS, Shenyang 110016, PR China
b College of Land and Environment, Shenyang Agricultural University, Shenyang 110161, PR China
c Institute of Soil Science and Soil Geography, University of Bayreuth, 95440 Bayreuth, Germany

Received 16 October 2002; received in revised form 29 January 2003; accepted 30 January 2003

Abstract

Storage of soil organic matter (SOM) in earthworm casts contributes to an improvement of soil fertility, especially in the tropics. This study was conducted to elucidate the incorporation of microbe- and plant-derived organic compounds into earthworm casts in humid Laos. Composite samples of earthworm casts and surrounding topsoil (0–10 cm) were fractionated into the following size fractions: coarse sand (250–2000 μm), fine sand (20–250 μm), silt (2–20 μm), and clay (<2 μm). Thereafter, lignin-derived phenols, neutral, acidic, and amino sugars were determined in bulk samples and the fractions. Organic C and N were enriched in earthworm casts, exceeding the respective C and N contents of the surrounding soil by a factor of 1.5, and 1.3, respectively. This enrichment was apparent in all particle-size fractions, and was not restricted to certain organic compounds. Plant-derived lignin, however, was accumulated in preference to microbe-derived amino sugars. The latter were rather redistributed among different size fractions of the casts. As differences in SOM composition between casts and surrounding soil were reflected most clearly for the silt fractions, it is concluded that organic matter storage in the casts of humid Laos is accompanied by the incorporation of SOM into (pseudo) stable silt-sized microaggregates.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Lignin; Sugar; Endogeic earthworm; Particle-size fractionation; Soil; Tropics

1. Introduction

The activity of endogeic earthworms in the humid tropical environment accelerates initial soil organic matter (SOM) turnover, which is followed by the production of organic matter- and nutrient-rich casts. The formation of casts may enhance storage of nutrients because mineralization of SOM from casts may be later blocked for several moths (see Lavelle and Martin, 1992). Furthermore, a recent study indicated that organic matter in the casts, once stabilized (and left undisturbed by earthworms), can maintain this stabilization for many years (Mcinerney and Bolger, 2000). This stabilization was thought to be due to physical protection of organic matter in the cast aggregates (Martin et al., 1992). Nevertheless, chemical mechanisms may also contribute to the stabilization because evidence shows that the casts are held together by strong interactions between mineral soil particles and SOM that is enriched in bacterial polysaccharides and fungal hyphae (Bhandari et al., 1967; Domsch and Banse, 1972). Little is known, however, about the fate of such bacterial and fungal residues in the casts.
Guggenberger et al. (1996) showed that earthworm casts were enriched in both plant- and microbe-derived saccharides as well as in intact lignin compared to semi-arid savanna soils of Columbia. Particle-size fractionation revealed that this enrichment of sugars and lignin was restricted to sand-sized SOM, being particulate in nature. Saccharides and lignin in mineral associated SOM of clay and silt fractions were not different in the earthworm casts compared to the surrounding savanna soil (Guggenberger et al., 1996). The authors concluded that fresh, labile SOM was incorporated within the casts and physically protected from decay in the semi-arid soil environment. Fragoso et al. (1993) also found for soils in humid climates that earthworms concentrated SOM in cast fractions >50 μm, but there was no further information about associated lignin and carbohydrates.

The degree of possible lignin and carbohydrate inclusion in particle-size fractions of earthworm casts in humid climates (similar to semi-arid savanna; Guggenberger et al., 1996) has not yet been evaluated to our knowledge. It is also unclear whether fungal or bacterial saccharides contribute to the larger pool of microbe-derived sugars in the sand-sized SOM of the casts. This cannot be generalized from previous studies, since fungal species may be differently affected during passage through the earthworm gut (Moody et al., 1996). Amino sugar analyses may help to differentiate between the average of bacterial and fungal residues in soil (Parsons, 1981; Chantigny et al., 1997), i.e. it should help to elucidate the origin of microbial N in earthworm casts.

The objective of this study was to elucidate the incorporation of lignin-derived phenols, neutral, acidic, and amino sugars into particle-size fractions of earthworm casts in Laos.

2. Materials and methods

2.1. Site and soil

The site is located at Xiengnueun District, Luang Prabang Province, Laos, with slopes ranging from 25 to 35%. The climate is tropical monsoon, characterized by two distinctive seasons (dry and rainy), with annual precipitation of 1350 mm and annual temperature of 26 °C. The soil is classified as Typic Haplustalf. The field sampled was a 2-year fallow under tropical grass. Composite surface soil samples (0-10 cm) were taken at five locations within each of three 240 m² plots (in the same field) in the fall of 1999. Thereafter, (endo-geic) earthworm casts were separated by hand from surrounding soil. The age of the casts did not exceed 2 years since earthworms were rarely found before the fallow treatment. In addition, we collected three composite surface samples of neighboring traditional farmed sites, where the indicator crop was upland rice.

2.2. Fractionation

The bulk soil and earthworm cast samples were dry sieved to remove particles >2 mm, then fractionated into four size fractions, using the following procedure of Amelung et al. (1996a). Briefly, 30 g of fine soil (<2 mm) was treated ultrasonically at 60 J ml⁻¹ with a probe type sonicator (Heat Systems, model W 185 F) in a soil/water ratio of 1:5 to disperse macroaggregates (>250 μm). The maximum output of ultrasonic energy was 48.6 ± 2 W. The coarse sand fraction (250–2000 μm) was isolated by wet sieving. Final ultrasonic dispersion was applied again at 440 J ml⁻¹ to the <250 μm suspension in a soil/water ratio 1:10 to disperse the samples completely. Centrifugation was used to separate the clay fraction (<2 μm). Wet sieving was performed to separate silt (2–20 μm), and dry sieving for fine sand (20–250 μm). All fractions were dried at 40 °C and ground for chemical analysis.

The particle-size yields were similar for bulk soil and casts (Table 1).

2.3. Chemical analyses

The sub-samples of all size fractions were analyzed for total C and N with a C/N/H/S-analyzer (Elementar GmbH, Hanau, Germany). Since there was no inorganic C in the soil, the measured C represents organic C.

Amount and state of oxidative decomposition of lignin were estimated with lignin parameters obtained from alkaline CuO oxidation at 170 °C for 2 h (Amelung et al., 1997). Alkaline CuO oxidation releases phenols from the lignin macromolecule. Consequently, the sum of vanillyl (V), syringyl (S) and cinnamyl (C) phenolic CuO oxidation products (VSC) indicates a relative amount of lignin, the VSC-lignin.
Table 1
Texture and organic matter in particle-size fractions of the studied samples

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Fallow soil</th>
<th>Earthworm castsb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSD (%)</td>
<td>C (g kg(^{-1}) fraction)</td>
</tr>
<tr>
<td>Clay</td>
<td>34.0</td>
<td>39.1</td>
</tr>
<tr>
<td>Silt</td>
<td>39.2</td>
<td>14.5</td>
</tr>
<tr>
<td>Fine sand</td>
<td>16.8</td>
<td>28.0</td>
</tr>
<tr>
<td>Coarse sand</td>
<td>10.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

a PSD: particle-size distribution as estimated by particle-size yields after fractionation (%); E\(_C\) = (g C kg\(^{-1}\) fraction)/(g C kg\(^{-1}\) soil).
b C and N contents are significantly higher in the casts compared with those in the fallow soil (P < 0.05).

Real lignin contents cannot be determined. The phenolic products were derivatized with a 1:1 mixture of pyridine and bis-trimethylsilyl-trifluoroacetamide (Fluka), separated by capillary gas chromatography (HP 5890 gas chromatograph, HP Ultra 2 fused silica column) and detected by a flame ionization detector. Ethylvanillin was added as an internal standard prior to CuO oxidation, and phenylacetic acid before derivatization to determine the recovery of ethylvanillin.

Determination of sugars followed the method of Amelung et al. (1996). Briefly, neutral sugars and uronic acids were released from soil with 4 M trifluoroacetic acid at 105 °C for 4 h. After purification with XAD-7 and Dowex 50 W X8 resins, trimethylsilyl oxide derivatives were prepared by the method of Andrews (1989) with slight modification. Identification and quantification of the oximes was performed by gas-liquid chromatography (same instrumentation as for lignin-derived phenols). Myo-inositol was added as an internal standard prior to hydrolyses, 3-O-methylglucose served as a second internal standard to control for the recovery of myo-inositol.

Amino sugars were determined according to the method of Zhang and Amelung (1996). After hydrolysis with 6 M HCl for 8 h, the solution was filtered, adjusted to pH 6.6–6.8 and centrifuged and freeze-dried. Methanol was used to wash amino sugars out from the residues. Amino sugars were transformed into aldononitrile derivatives. Excess anhydride was destroyed with 1 M HCl and water, after the amino sugar derivatives were extracted with dichloromethane from the aqueous solution. Gas chromatographic separation of the amino sugar derivatives was carried out with the same instrumentation as mentioned above. Myo-inositol was added as an internal standard before derivatization, and methyl-glucamine as the recovery standard before derivatization.

2.4. Statistical analysis

The data were analyzed by one-way analysis of variance along with post hoc separation of means by the LSD procedure to compare parameter values between earthworm casts and the surrounding soil. Mean values of the various parameters are presented ± standard errors (SEs) in the text.

3. Results and discussion

After particle-size fractionation, on average 100% of C, 104% of N, 104% of amino sugars, 98% of lignin-derived phenols, and 95% of neutral sugars were recovered relative to the corresponding yields in the bulk soil. This suggests that there were no significant losses of these compounds during size fractionation. Apart from the C/N ratio that tended to be lower in the coarse than in the fine sand fraction (Table 1), the distribution pattern of individual compounds in the size fractions is similar to earlier observations for soils in temperate (Zhang et al., 1998), and tropical climates (Amelung et al., 1998b). The standard error of C and N measurements was less than 5% and that of others less than 10% of the mean, with the exceptions of muramic acid and mannosamine, where the SE sometimes exceeds 10%, but was not greater than 15% (at very low concentration).
3.1. Carbon and nitrogen

The earthworm casts contained $40.8 \pm 2.5 \text{ g kg}^{-1}$ C and $3.5 \pm 0.19 \text{ g kg}^{-1}$ N, compared to only $26.1 \pm 1.7 \text{ g kg}^{-1}$ C and $2.8 \pm 0.16 \text{ g kg}^{-1}$ N in the bulk soil under fallow. The traditional farmed sites contained only $22.0 \pm 1.6 \text{ g}$ C and $2.4 \pm 0.18 \text{ g N kg}^{-1}$ soil. Whereas a 2-year fallow period improved SOM levels by 15% of the original C and 9% of original N-values, the casts thus comprised SOM spots where SOM was accumulated by a factor of almost 2 compared to the traditional farming sites. This confirms earlier findings that SOM can be tremendously enriched in casts compared to bulk soil (Guggenberger et al., 1996). SOM accumulation was found in all size fractions (Table 1); however, the SOM gain was most pronounced for fine sand-sized SOM, which contained almost twice as much C and N in the casts as in the surrounding soil. Consequently, the C enrichment factor ($[E_C = \frac{g \text{ C kg}^{-1} \text{ fraction}}{g \text{ kg}^{-1} \text{ bulk soil}}]$) was higher in the fine sand fraction of the casts than in that of the surrounding soil. This might be attributed to the incorporation and physical stabilization of sand-sized, particulate plant residues in the casts (Guggenberger et al., 1996).

3.2. Lignin

The sum of lignin-derived phenols (VSC) averaged $31 \pm 2.9 \text{ g kg}^{-1}$ C in the casts compared to $17 \pm 1.8 \text{ g kg}^{-1}$ C in the surrounding topsoil (difference significant at $P < 0.05$). Lower acid-to-aldehyde ratios ([ac/al]) for the vanillyl (V) and the structural units indicated that the lignin component was less oxidized in the casts. This can be reconciled with the results of Guggenberger et al. (1996) who found that earthworm casts in Columbia were enriched in less-oxidized lignin. We could not confirm, however, that the less pronounced side-chain oxidation was related to physical protection of plant residues as was probably the case in the study of Guggenberger et al. (1996). Instead, the lignin component of the clay and silt fractions in particular showed less evidence of side-chain oxidation in the casts than in the surrounding topsoil (Fig. 1). The acid-to-aldehyde ratios of the two sand fractions were little affected (data not shown). Possibly initial breakdown of little-oxidized lignin by earthworm activity might have resulted in a more pronounced input of little altered SOM to the clay fractions.

3.3. Neutral and acidic sugars

There were no clear differences in neutral sugar concentrations and patterns between earthworm casts and surrounding soil for all fractions (Table 2), confirming previous findings from semi-arid climates that sugars were less affected by earthworm activity than lignin (Guggenberger et al., 1996). When considering, however, only the coarse sand fraction, neutral (and acidic) sugar concentrations tended to be lower in the casts compared to the fallow soil ($P < 0.05$; Table 2). A higher ratio of galactose and mannose (microbe derived) to xylose and arabinose (plant derived; Cheshire, 1979) indicates that this is attributable to decay. As coarse sand-sized SOM is least altered among fractions, neutral sugar analysis confirms, therefore, that earthworm activity results in a very initial alteration of SOM (Lavelle and Martin, 1992).

In contrast to neutral sugar concentrations, acidic sugars tended to be preferentially enriched in the SOM of the $<250 \mu m$ fractions (Table 2). This might result from the production of exopolysaccharides, being rich in polyuronic acids (Schlegel, 1992).
3.4. Amino sugars

Amino sugars are not synthesized by plants or by earthworms in significant amount (Parsons, 1981). They may be used, therefore, to mark microbial residues in the soil environment. The amino sugar concentrations in the casts averaged 60 ± 5.2 g kg⁻¹ C, whereas in the surrounding soil 99 ± 8.3 g kg⁻¹ C were found (difference significant at $P < 0.05$). The SOM of earthworm casts was, therefore, depleted in microbial residues. This corresponds with higher proportions of non-microbe-derived SOM, such as lignin (see above). Particle-size fractionation revealed, however, that a depletion of amino sugars in the earthworm casts was caused by lower amino sugar accumulation in the clay fraction (Table 2), whereas there is no opposite trend for the plant-derived lignin (Fig. 1). We conclude that lower amino sugar concentrations in the casts were also caused by less amino sugar production. This might be due to inhibited microbial activity after initial aging of the casts, especially in dry seasons of the monsoon climate in Laos (Lavelle and Martin, 1992).

Dilution effects of plant-derived C on amino sugar concentrations can be eliminated when amino sugars are related to N rather than to C. For the bulk soil the trend remained, i.e. the casts were depleted in amino sugar N proportions, containing 600 ± 66 g amino sugars kg⁻¹ N compared with 920 ± 85 g amino sugars kg⁻¹ N in the surrounding topsoil. When considering the amino sugar distribution among size fractions, a different picture is obtained. In the surrounding soil, there was a secondary enrichment of amino sugars in the fine sand fraction that disappeared in the casts (Table 2). Obviously, there was a redistribution of amino sugars from fine sand to silt when soil material was aggregated in the casts (Fig. 2). Usually, interactions between microbial saccharides with (especially silt-sized) aggregates are very stable in soil (Tiessen et al., 1984; Lynch and Bragg, 1985). It remains to be shown, therefore, whether higher relative proportions of amino sugars in the silt fraction contribute to high aggregate stability of casts compared with that of aggregates in the surrounding soil (Lal and Akinremi, 1983; Lavelle and Martin, 1992).

Different microorganisms produce different amino sugars. Whereas muramic acid is uniquely produced by bacteria, glucosamine is common in fungal and microarthropod chitin (Kenne and Lindburg, 1983; Parsons, 1981). The origin of galactosamine is less clear, however, Sowden and Ivarson (1974) showed that little if any galactosamine was found in fungi inoculated incubation experiments, whereas it was synthesized by bacteria. Consequently, both ratios of glucosamine to muramic acid and of glucosamine to galactosamine were used to differentiate different contributions of
The ratios of glucosamine to muramic acid and of glucosamine to galactosamine were almost identical for the bulk samples. The glucosamine to muramic acid ratio averaged 22 and 23 for the casts and bulk soil, while the corresponding glucosamine-to-galactosamine ratios were 2.2 and 1.9. We conclude that formation of earthworm casts did not result in different accumulation of fungal and bacterial residues. Nevertheless, when considering the redistribution of amino sugar N proportions among fractions, it is evident that the glucosamine-to-muramic-acid ratio followed the same trend as the sum concentrations. Apparently, the shift of amino sugars from the fine sand to silt fraction was mainly caused by a shift in chitin-derived glucosamine. Possibly fungal hyphae, associated with fine sand-sized SOM (Tisdall and Oades, 1982) were broken down during the gut passage of the soil material, ending up as smaller pieces associated with silt-sized particles.

4. Conclusions

In humid Laos, whereas 2-year fallow only slightly increased SOM levels, earthworm casts were considerably enriched in SOM, and, thus, in total lignin and sugar contents. The SOM accumulation was not restricted to sand-sized SOM, but it was maintained by all size fractions. The SOM composition was less affected. Higher concentrations of less-oxidized lignin in SOM of finer fractions suggested a more preferential accumulation of plant-derived materials in primary particles in casts than in those of the surrounding soil. As SOM release from minerals controls SOM turnover (Haider, 1992), we conclude that apart from physical stabilization of SOM in casts (Lavelle and Martin, 1992), SOM storage in casts is also favored by chemical stabilization processes. Higher SOM storage in fallow plots might be largely attributed, therefore, to SOM-retaining earthworm activity.

Due to the preferential accumulation of plant-derived SOM in the casts, their SOM was relatively depleted in microbe-derived compounds, such as amino sugars, when compared to the SOM of the surrounding soil. In the casts, however, more amino sugars were attached to the mineral matter, where they are known to be more effective in binding small particles to larger, forming stable aggregates.

Acknowledgements

Xudong Zhang is involved in the “Hundred Talents Program” of the Chinese Academy of Sciences and the research is partly funded by the program. We thank Adisak Sajjapongse, and Chalinee Niamskul at the International Board for Soil Research and Management (IBSRAM), Bangkok, Thailand for their help with soil sampling.

References

Amelung, W., Zech, W., Zhang, X., Sajjapongse, A., Niamskul, C., 1998b. Lignin and carbohydrates in soils under secondary...
forest, alley cropping and continuous farming, Thailand. Z.
of monosaccharides: improvements and comparisons using
trifluoroacetylation and trimethylsilylation of sugar O
Bhandari, G.S., Ranghawa, N.S., Maskina, M.I., 1967. On the
polysaccharide content of earthworm casts. Curr. Sci. 36, 519–
520.
Chantigny, M.H., Angers, D.A., Privéot, D., Vezina, L.-P.,
Chalifour, F.P., 1997. Soil aggregation and fungal and bacterial
biomass under annual and perennial cropping systems. Soil Sci.
Fragoso, C., Barrios, I., González, C., Arteaga, C., Patrón, J.C.,
1993. Relationship between earthworms and soil organic
matter levels in natural and managed ecosystems in the
Mexican tropics. In: Mulongoy, K., Merckx, R. (Eds.), Soil
Organic Matter Dynamics and the Sustainability of Tropical
Agriculture. Proceedings of an International Symposium in
Guggenberger, G., Thomas, R.J., Zech, W., 1996. Soil organic
matter within earthworm casts of an anecic–endogeic tropical
Haider, K., 1992. Problems related to the humification processes
Kempe, L.K., Lindberg, B., 1983. Bacterial polysaccharides. In:
Lal, R., Akkremi, O.O., 1983. Physical properties of earthworm
casts and surface soil as influenced by management. Soil Sci.
135, 114–122.
Lavelle, P., Martin, A., 1992. Small-scale and large-scale effects
of endogeic earthworms on soil organic matter dynamics in
organic matter assimilation by a geophagous tropical earthworm
Munnecke, M., Bolger, T., 2000. Decomposition of Quercus
petraea litter: influence of burial, composting and earthworms.
Hruby, R.A., Peretz, T.G., Dighton, J., 1996. Fate of some fungal
spores associated with wheat straw decomposition on passage
through the guts of Lumbricus terrestri and Aporrectodea. Soil
Biol. Biochem. 28, 533–537.
Parsons, J.W., 1981. Chemistry and distribution of amino sugars in
Schlegel, H.G., 1992. Allgemeine Mikrobiologie. Thieme-Verlag,
Stuttgart.
Sowden, F.J., Ivarson, K.C., 1974. Effects of temperature on
changes in the nitrogenous constituents of mixed forest litters
during decomposition after inoculation with various microbial
organic matter transformations in relation to organo-mineral
Zhang, X., Amelung, W., 1996. Gas chromatographic
determination of muramic acid, glucosamine, galactosamine,
signature of particle size fractions in soils of the native prairie