Tagungsbeitrag zu: DBG-Tagung
Titel der Tagung: „Böden verstehen - Böden nutzen - Böden fit machen“
DBG, 3. - 9. September 2011, Berlin
Berichte der DBG (nicht begutachtete online Publikation)
http://www.dbges.de

Sterols in soil organic matter of sandy arable soils: Quantification using mass spectrometry and their relation to mineralizability of soil organic N

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Abstract

Lately, a significant negative correlation between proportions of the compound class of sterols from pyrolysis-field ionization mass spectrometry (Py-FIMS) and net N mineralizability of soil organic N was found. However, main plant sterols (β-Sitosterol, Stigmasterol, and Campesterol) cannot be clearly verified and quantified with Py-FIMS, and there are only very few studies on measuring concentrations in soils. Thus, the objective was the extraction, identification and quantification of typical plant sterols and their relation to net N mineralization rates. The three sterols were identified and quantified in lipid extracts (Soxhlet procedure) using gas chromatography - mass spectrometry (GC-MS). Concentrations were similar to few other available studies, but concentrations of the three sterols were not significantly correlated with net N mineralizability. As quantification was difficult due to co-elution, further optimization of the methodology is necessary. In addition, the underlying mechanisms also need to be clarified.

Keywords: β-Sitosterol, Stigmasterol, Campesterol, GC-MS, long-term lab incubation

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1 Introduction and objective

Many sandy arable soils of NW-Germany show relatively high soil organic matter (SOM) contents (8 - 59 mg kg^{-1}), but rather low mineralizability of organic N (Heumann et al. 2002) and C (Springob & Kirchmann 2002). For nine of these soils (clay < 50 mg kg^{-1}, organic C ranging from 11 - 52 mg kg^{-1}) we found a highly significant negative correlation between the long-term net N mineralization rates and the proportions of the compound class of sterols (in % of total ion intensity, TII) from pyrolysis-field ionization mass spectrometry (Py-FIMS, see M+M) (Fig. 1; Heumann et al. 2011). The correlation was even closer with the attributed TII proportions of only β-Sitosterol, and slightly less close for Stigmasterol and Campesterol. However, these typical main plant sterols (e.g. Otto and Simpson 2005) cannot be clearly verified and quantified with Py-FIMS, and there are only very few studies on measuring concentrations of certain sterols in soils. Thus, our objective was the extraction, identification and quantification of typical plant sterols and their relation to net N mineralization rates.

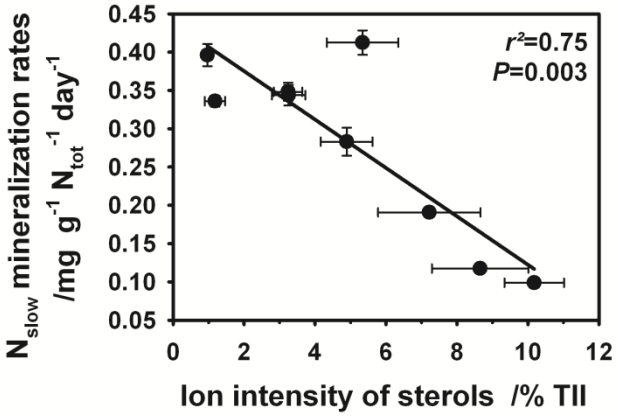

Fig.1: Ion intensity of sterols (Py-FIMS) *vs.* net N mineralizability (from Heumann et al. 2011)

2 Material and methods

Sites and soils

- six sandy, mostly podzolized arable soils (Tab. 1) from Northern Germany, that have showed the closest correlation in Fig. 1 (regression line from Heumann et al. 2011)

Tab 1:

sample no.	C_{org} mg kg^{-1}	N_{tot} mg kg^{-1}	C:N	soil type (FAO)
S2	14	0.96	15	Gleyic Podzol
S4	21	0.99	21	(Stagni-)Haplic Podzol
S5	32	1.79	18	(Stagni-)Haplic Podzol
S7	52	2.26	23	Gleysol
S8	22	0.97	23	Gleyic Podzol
S9	35	1.02	34	Gleyic Podzol

Mineralizability of organic N

- long-term laboratory incubations over 200 days were used to determine two N pools (Heumann et al. 2002):

$$N_{\min}(t) = N_{fast}(1-e^{-k_{fast}t}) + N_{slow}(1-e^{-k_{slow}t})$$

- mean net N mineralization rates of pool N_{slow} relative to soil organic N were defined as mineralizabilty of soil organic N

Py-FIMS

- the molecular-chemical composition of SOM was analyzed in 3 replicates
- detected m/z-signals were amalgamated to compound classes (Schulten and Leinweber 1999)

Sterol extraction, identification and quantification via GC-MS

- Soxhlet-extraction (= well-established methodology after Jansen et al. 2006): 50g air-dried soil (Wt_{SOI}) was extracted by boiling in 250ml of a 93:7 (vol/vol) mixture of the organic solvents Dichloromethane and Methanol for 24 h. Lipid extracts (sterols are lipids) were cooled and dried using a rotary evaporator.
- GC-MS measurements: Dried extracts were measured after trimethylsilylation with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) on a 60 m HP5-MS column (0.25 mm internal diameter, 0.25 µm film thickness, J&W Scientific, USA). Here, the sterane 5(α)-androstane was used as an internal standard (*is*). For quantification, separate measurements were done for standards (*StS*) of the three main plant sterols β-Sitosterol, Campesterol, and Stigmasterol (HPLC-grade; Chromadex, USA) together with the internal standard (*IS*) in order to determine the relative response factors (*RRF*) of the three sterols. Identification was done using the libraries NIST08 and Wiley.

$$RRF = \frac{P_{IS}}{Wt_{IS}} \frac{Wt_{StS}}{P_{StS}}$$

P: peak area
Wt: weight (for GC-MS measurement)
StS: sterol standards
IS: internal standard (Androstane), measured with StS

$$Wt_x = RRF \frac{P_x}{P_{is}} Wt_{is}$$

x : substance x (here: certain sterol)
is: internal standard (Androstane), measured with extracts

$$C_x = \frac{Wt_x}{Wt_{soil}}$$

C_x: concentration of substance x (sterol) in the soil

Statistics

- Spearman's rang correlation coefficient r was determined for correlations, since it is independent from the distribution

3 Results and discussion

Concentrations of lipid extracts

The concentrations of lipid extracts (Tab. 2; per g soil and per g C_{org}) varied strongly, but were in the same range as reported elsewhere (Wiesenberg et al. 2004, 2006).

Tab.2:

	Lipid extract mg g^{-1} soil	Lipid extract mg g^{-1} C_{org}	Campesterol µg g^{-1} soil	Stigmasterol µg g^{-1} soil
S2	0.76	54.0	0.21	0.30
S4	0.23	11.0	0.03	0.04
S5	0.44	13.8	0.20	0.09
S7	0.18	3.5	0.06	0.08
S8	0.45	20.3	0.18	0.14
S9	1.33	38.0	0.06	0.04

	β-Sitosterol µg g^{-1} soil	net N mineralizability mg N_{slow} per % of N_{tot}	sterols (Py-FIMS) % of total ion int.
S2	0.54	0.348	1.9
S4	0.13	0.285	3.3
S5	0.65	0.191	4.8
S7	0.21	0.396	0.7
S8	0.64	0.117	5.6
S9	0.28	0.099	6.3

Concentrations of three main plant sterols

Concentrations of β-Sitosterol were comparable to few similar studies (Jansen et al. 2006; Otto and Simpson 2005). The three sterols together amounted to less than 1 %

of the total lipid extracts (Tab. 2).
However, quantification was somewhat difficult due to co-elution in the chromatograms. Here the peaks were split, but optimizations (choice of columns, heating procedure) might further improve the quantification.

Correlations
Concentrations of single sterols as well as of the sum of the three studied sterols were not significantly correlated to net N mineralization rates per g of soil or per g of total N (Fig. 2), to C_{org}, lipid extracts or sterol intensities from Py-FIMS.

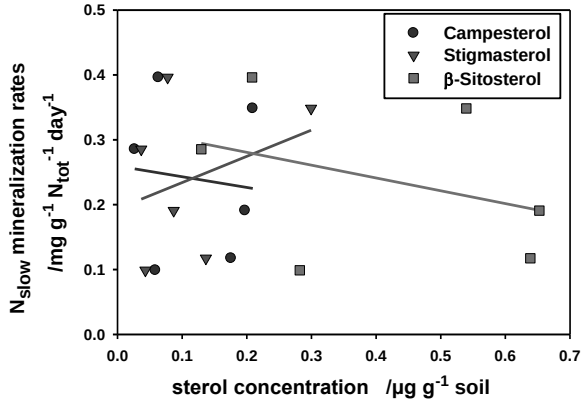

Fig.2: Soil concentrations of three plant sterols (GC-MS) *vs.* net N mineralizability ($P > 0.05$)

This is in contrast to earlier results when sterols were estimated by Py-FIMS (Fig. 1) which could possibly be due to the incomplete extraction of ester- und etherlinked sterols (Otto and Simpson 2006), whereas Py-FIMS also detects these compounds.
There were significant correlations only within single concentrations of quantified sterols (Fig. 3).

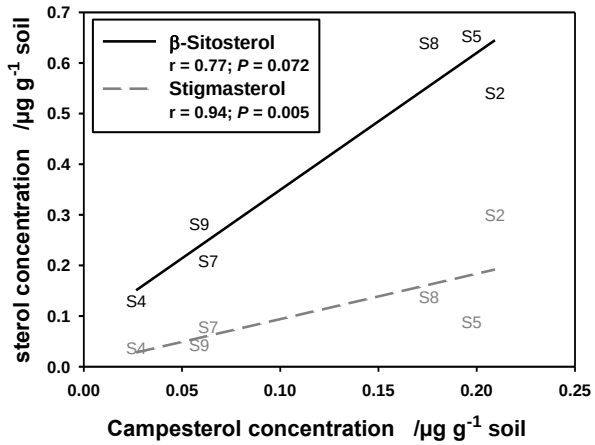

Fig.3: Correlations between soil concentrations of three different plant sterols from GC-MS

4 Conclusions

Via GC-MS the three main plant sterols β-Sitosterol, Campesterol and Stigmasterol were identified and concentrations in the soil quantified. However, there were no significant correlations between sterol concentrations and net N mineralization. This could be due to incomplete extraction as well as to co-elution in the chromatograms. Thus, further optimization of the methodology is necessary for more accurate quantification. The underlying mechanisms also need to be clarified.

Acknowledgements

The authors wish to thank Ulrike Pieper and Silke Bokeloh for their dedicated work in the laboratory. The study was funded in part by *DAAD 50107117, British Council-ARC*, and *"Deutsche Bundesstiftung Umwelt"*. The Mass Spectrometry Laboratory at the Faculty of Agricultural and Environmental Science, University of Rostock, was funded by the *"Exzellenzförderprogramm"* of the *Ministerium für Bildung, Wissenschaft und Kultur Mecklenburg-Western Pomerania, project UR 07 079*.

References

Heumann S, Böttcher J & G Springob. 2002. N mineralization parameters of sandy arable soils. J Plant Nutr Soil Sci 165, 441-450.
Heumann S, Schlichting A, Böttcher J, & P Leinweber. 2011. Sterols in soil organic matter in relation to nitrogen mineralization in sandy arable soils. J Plant Nutr Soil Sci 174, 576–586.
Jansen B, Nierop KGJ, Kotte MC, de Voogt P & JM Verstraaten. 2006. The applicability of ASE to extract lipid biomarkers from soils. Appl Geochem 21, 1006-1015.
Otto A & MT Simpson. 2005. Degradation and Preservation of Vascular Plant-Derived Bio-markers in Grassland and Forest Soils from Western Canada. Biogeochem 75, 377-409.
Otto A & MT Simpson. 2006. Sources and composition of hydrolysable aliphatic lipids and phenols in soils from western Canada. Org Geochem 37, 385-407.
Schulten H-R & P Leinweber. 1999. Thermal stability and composition of mineral bound organic matter in density fractions of soil. Eur J Soil Sci 50, 237-248.
Springob G & H Kirchmann. 2002. C-rich sandy Ap horizons of specific historical land-use contain large fractions of refractory organic matter. Soil Biol Biochem 34, 1571-1581.
Wiesenberg GLB, Schwark L & MWI Schmidt. 2004. Improved automated extraction and separation procedure for soil lipid analysis. Eur J Soil Sci 55, 349-356.
Wiesenberg GLB, Schwark L & MWI Schmidt. 2006. Extractable lipid contents and colour in particle-size separates and bulk arable soils. Eur J Soil Sci 57, 634-643.