Sensitivity of soil organic matter pools decomposition to microbial activity and temperature: a 13C and 14C tracer study

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1. Purpose of visit

Decomposition and turnover of soil organic matter (SOM) are key processes responsible for carbon (C) sequestration in soils, which maintain the main C stock in terrestrial ecosystems. Two global change components may strongly affect on the C stock in soil and its turnover: increasing temperature and increasing atmospheric CO_2 (Buchmann 2000; Wiesenberg et al. 2008). To get a clearer understanding of how these factors influence on the SOM turnover, we need detailed information about the processes of SOM transformation. To investigate these processes an experiment with C and N labeled low molecular weight organic substances (LMWOS) was made. These substances play the major role regarding SOM turnover, as LMWOS are one of the basic step, which nearly each C atom passes during degradation of plant and microbial macromolecules. Whereas degradation of macromolecules is mainly carried out by exoenzymes the main use of LMWOS is carried out by microbial cells (Dippold, in preparation), which either incorporate the C into their microbial biomass or oxidize it to CO_2 and thus contribute to increasing atmospheric CO_2. Especially with regard to changing soil microbial community due to changing soil temperature, a focus on the role of single microbial groups in the utilization of LMWOS must be done.
In a first step, the use of three major substance classes (amino acids, sugar monomers and carboxylic acids) was made. Therefore C and N labelled members of theses substance classes were injected in soil columns installed in a field experiment. After 3 and 10 days columns were sampled and bulk C and N content, uptake of tracer into the microbial biomass (by chloroform fumigation-extraction) and incorporation of C into specific microbial groups (by C analysis of phospholipid fatty acid (PLFA)) were determined.
By this experiment three major questions of LMWOS transformations could be answered:

1. which microbial groups of agroecosystems are most efficient in the utilization of C from LMWOS?
2. do specific microbial groups differ in their uptake of LMWOS substance classes (sugar monomers, amino acids and carboxylic acids)?
3. do specific microbial groups differ in the uptake of single compounds of one LMWOS-substance class:
   - → amino acids: neutral (alanine) vs. basic (glutamic acid)
   - → sugars: hexose (glucose) vs. pentose (ribose)
   - → carboxylic acid: short chain (acetic acid) vs. long chain (palmitic acid)

2. Description of the work carried out during the visit

Samples for investigation of LMWOS transformation were taken from a field experiment, carried out near Bayreuth. The field experiment had a randomized block design with the 4 blocks giving 4 field repetitions, for each of the 6 substances 28 columns (7 sample times x 4 repetitions) were labeled. Labelling was performed in August 2010 by injecting the 13C and 15N-labelled compounds into the soil columns, which were installed to a depth of 10 cm in soil. For PLFA-analysis samples from the first two sampling times (3 days and 10 days) were chosen. The samplings columns were completely removed from soil, volume, weight and water content determined, sieved to 2 mm and soil was stored frozen (- 20ºC) until analysis.
While bulk measurement of soil samples and C/ N of microbial biomass had been already done, I was responsible for PLFA-analysis of these samples.

The main procedures of PLFA analysis:

- extraction
Four field replications of each samples of 6 g of fresh soil were extracted two times with a one-phase solvent extractant which was a mixture of chloroform, methanol and citric acid 1:2:0,8 (v/v). Polar compounds were separated from the lipid compounds by liquid-liquid-extraction. 19:0-phospholipid was used as first internal standard, to show the recovery of all investigated phospholipids (Frostegard et al., 1991).

- purification
The phospholipids were separated from neutral and glycolipids using solid-phase extraction columns (1 ml of activated Silicagel; Merk., mesh 70-250 μμ size) by elution with 5 ml of chloroform, followed by 20 ml of acetone. Phospholipids were then eluted with 20 ml of methanol, and dried under N_2 (Frostegard et al., 1991).

- derivatization
PLFA-fraction was then subjected to alkaline saponification and free fatty acids were methylated with BF_3 in methanol. Fatty acid methyl esters (FAMEs) were extracted three times with 1 ml hexane. Combined hexane aliquots were dried under N_2, and re-dissolved in toluene with addition of the second internal standard (13:0 FAME at 1 mg/ml) (Knapp,1979).

- *pre-measurement on GC-MS*.
All samples were analysed using a Hewlett Packard 5890 gas chromatograph coupled with mass-selective detector 5971A. The 25 m HP-1 methylpolysiloxane column was used. A single 1 μl injection was analysed with an initial temperature of 80 ºC and then ramped to 164 ºC at 10 ºC/min , than to 230 ºC at 0,7 ºC/min, than to 300ºC at 10ºC/min at a constant flow rate of 2,4 ml/min. Peaks were identified and quantified using calibration curve which was built using 29 standard substances in six different concentrations.

- *final measurement on IRMS*:
To get exact isotope ratios of the single fatty acids GC-C-IRMS-measurement are running currently on a Delta PlusTM (Thermo Finnigan, Bremen, Germany) coupled to a gas chromatograph (GC; Trace GC 2000, Thermo Finnigan) via a combustion interface. Detailed info of the instrumental setup and the used GC-column can be found in Sauheitl et al. (2005). Online referencing of $\delta^{13}C$ values will be done by the injection of several reference gas pulses directly into the IRMS during measurement as described in Glaser and Amelung (2002). Measured δ-values of PLFAs will be corrected for the influence of derivative C in analogy to Glaser and Amelung (2002) and ^{13}C enrichment in single PLFAs will be calculated as the difference between labelled and unlabelled soil samples. The amount of tracer-C uptake into PLFA will be calculated based on the ^{13}C enrichment of PLFAs, following a two pool dilution model (Gearing, 1991).

3. ***Description of the main results obtained***
Microbial fingerprint of the investigated agroecosystem was compared with the grassland ecosystem (Sauheitl et al., submitted) and a spruce forest ecosystem (Heinrich et al, in preparation). Mean percents of total PLFA-content from each of these ecosystems are shown in Table 1.

Table 1. Mean percent of total phospholipid fatty acid content

fatty acid	agricultaral soil		grassland soil		spruce forest soil	
	mean % of total plfa	std-error	mean % of total plfa	std-error	mean % of total plfa	std-error
i14:0	1,86	0,27	n. m.	n. m.	n. m.	n. m.
a14:0	0,66	0,09	n. m.	n. m.	n. m.	n. m.
14:1w5c	0,28	0,03	n. m.	n. m.	n. m.	n. m.
14:0	1,96	0,15	1,27	0,04	2,85	0,12
i15:0	9,29	0,70	4,98	0,40	9,69	0,94
a15:0	6,93	0,48	3,32	0,27	2,67	0,12
15:0	0,05	0,03	1,15	0,08	0,75	0,07
i16:0	2,29	0,11	2,31	0,06	5,03	1,34
16:1w7c	8,33	0,25	7,40	0,09	4,66	0,78
16:1w5c	3,56	0,29	7,31	0,21	1,85	0,37
16:0	17,21	0,77	14,36	0,23	13,77	0,95
10Me16:0	4,20	0,23	7,10	0,87	6,28	1,51
i17:0	1,35	0,10	1,40	0,10	1,14	0,09
a17:0	0,00	0,00	1,38	0,06	1,29	0,10
cy17:0	2,64	0,23	1,97	0,10	2,43	0,38
17:0	0,00	0,00	2,02	0,20	0,96	0,21
10Me17:0	n. m.	n. m.	0,35	0,03	0,96	0,17
18:2w6,9	0,29	0,18	5,43	0,63	2,93	0,10
18:1w9c	10,36	0,76	10,37	0,58	7,38	0,91
18:1w7c	17,94	0,33	13,23	0,26	11,36	2,38
18:0	2,33	0,27	4,61	0,15	3,34	0,07
10Me18:0	3,25	0,23	1,57	0,05	0,68	0,08
cy19:0	4,34	0,39	5,66	0,32	15,10	1,35
20:4w6c	0,77	0,23	2,81	0,95	4,88	0,70
20:1w9c	0,08	0,01	n. m.	n. m.	n. m.	n. m.
20:0	0,05	0,03	n. m.	n. m.	n. m.	n. m.

These results show, that those gram positive bacteria being represented by branched fatty acids with 15 and 16 C atoms had higher amount in agricultural and spruce soils than in grassland soil, whereas i17:0 and a17:0 had highest relative amount under grassland. The w7-fatty acids, being characteristic for gram negative biomass, dominated in the agricultural soil investigated in this study. The actinomyces biomass showed no consistent trend between the ecosystems which might be explained by different taxonomic structure of those

microorganisms. Protozoa was very low in the investigated agroecosystem as microbial biomass (data not presented here) was lowest in this ecosystem compared to forest and grassland. Fungi were also lowest in the agroecosystem because this ecosystem had lowest input of plant debris and pH was highest in this ecosystem. Vesicular arbuscular mycorhiza (VAM) was highest in grassland soils but it had also relevant amounts in agricultural soils. This agrees with the common view, that this type of symbiosis is characteristic for agricultural ecosystems but less relevant in forests.
In addition a PCA of the microbial fingerprint was conducted according to Sauheitl et al (submitted). PCA revealed three factors explaining 89% of the variance of the datasets. Factor loadings are shown in Table 2, each loading higher than 0.65 is marked in red. Factor loadings were used to separate PLFAs of the same microbial group but showing a different ecological pattern with respect to our datasets.

Table 2. Factor loadings of the representative fatty acids (FA)

Representative FAs	Factor 1	Factor 2	Factor 3
i14:0	-0,89	-0,38	0,05
a14:0	0,53	0,15	0,56
14:1w5c	-0,61	-0,70	0,03
i15:0	-0,97	-0,10	0,15
a15:0	-0,93	-0,20	0,30
i16:0	-0,57	0,79	0,04
i16:0	-0,38	-0,48	0,63
16:1w7c	0,92	0,02	0,14
16:1w5c	-0,18	-0,82	0,23
10Me16:0	0,20	0,95	-0,16
cy17:0	0,68	0,65	-0,02
18:2w6,9	0,12	0,15	-0,86
18:1w9c	0,92	0,24	-0,20
18:1w7c	-0,54	-0,52	0,46
10Me18:0	0,28	0,83	-0,27
cy19:0	0,74	0,49	-0,23
20:4w6c	0,08	0,17	-0,93
20:1w9c	-0,61	-0,70	0,03

The main microbial groups interpreted during this analysis and their percentage on total PLFA content is listed in Table 3. For these groups uptake of the LMWOS will be calculated and interpreted according to Sauheitl et al. (submitted) as soon as IRMS measurements will be finished.

Table 3. The relative amount of grouped representative fatty acids in soils

Name of group	Representative FAs	Relative amount (%)
Gram Positives	i14:0, i15:0, a15:0	17,9 ± 1,26
Gram Negatives group 1	16:1w7c, 18:1w9c, cy19:0, cy17:0	18,6±0,54
Gram Negatives group 2	14:1w5c,	0,3±0,03
VAM	16:1w5c	3,8±0,15
Actinomicees	i16:0, 10Me16:0, 10Me18:0	9,8±0,40
Fungi group 1	18:2w6,9,	1,1±0,26
Fungi group 2	20:1w9c	0,08±0,01
Protozoa	20:4w6c	0,8±0,23

The dominant groups in investigated soils were gram positives, gram negatives bacteria and actinomyces. The fungi and protozoa comprised less than 2 percent of microbial community, which could be connected with the features of agricultural land use and ecological conditions.

4. *Future collaboration with host institution*

After having a representative information about microbial use of LMWOS we will focus on the influence of temperature on the activity and structure composition of microorganisms which utilize the main classes of LMWOS. In addition, we will investigate which processes of LMWOS utilization will change with increasing temperature. Therefore an additional stay in Germany is planned and further studies are in preparation.

5. ***Projected publications***

These data (including the data which will result from IRMS measurement) are planned to be published in FEMS Microbiology Ecology at the end of the year during the next stay in Germany.

6. ***Other comments***

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