Determination of Substrate Quality Effects on Microbial-Derived Soil Organic Matter Using Compound-Specific Stable Isotope Analysis

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Introduction

Amino sugars (AS) are building blocks of the microbial cell wall and are stabilized in soil after cell death.

Fungal and bacterial cell walls are characterized by different AS signatures.

Amino sugars have been used to determine the contribution of fungal and bacterial residues to soil organic matter.

However, the dynamics, substrate effect and stabilization mechanisms of AS in soils are poorly understood, in part due to lack of adequate techniques to measure 'turnover rates' of AS.

Compound specific stable isotope analysis via gas chromatography combustion isotope ratio mass spectroscopy (GC-c-IRMS) is a potential tool for the determination of AS dynamics.

Hypotheses

More AS will be formed in treatments with more easily decomposable substrates.

Easily decomposable substrates favor production of bacterial rather than fungal AS.

In a sandy soil, biochemical recalcitrance will be the major stabilization mechanism for AS.

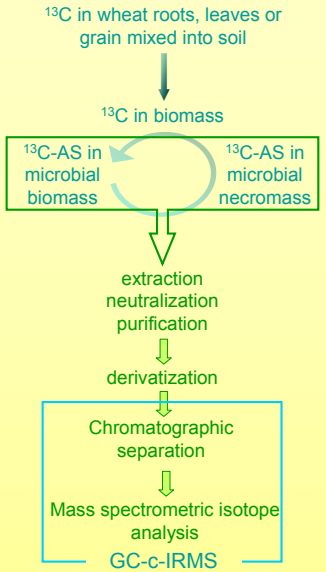
Objectives

Testing the feasibility of the GC-c-IRMS method to assess ^{13}C incorporation into soil AS.

Determining the effect of substrate quality on AS dynamics.

Enhancing the understanding of AS stabilization mechanisms by excluding physical stabilization due to aggregation (incubation in sandy soil).

Incubation & analysis



- δ ^{13}C of wheat is 800 ‰
- Decomposability grain > roots > leaves
- 14 weeks incubation
- soil: 90% sand
- AS analyses after 1, 2, 4, 8 and 14 weeks

Calculations

$$\delta^{13}C_{AS} = \frac{N_{Der}\delta^{13}C_{Der} - F - N_{acet}\delta^{13}C_{acet}}{N_{AS}}$$

$\delta^{13}C_{AS}$ is corrected $\delta^{13}C$ value of the AS

$\delta^{13}C_{Der}$ is measured $\delta^{13}C$ value of the derivatized AS

$\delta^{13}C_{Acet}$ is measured $\delta^{13}C$ value of acetic anhydride used for derivatization and measured by EA-IRMS ($\delta^{13}C$ = -24.38 ± 0.28‰, n=10)

N_{AS}, N_{Der} and N_{Acet} is the number of C-atoms in AS, the derivatized AS and in the added acetyl groups

F is a correction factor compensating for differences between EA-IRMS and GC-c-IRMS, for discrimination during derivatization and for concentration dependency

Reference: **Glaser, B., and S. Gross.** 2005. Rapid Communications in Mass Spectrometry 19:1409-1416.

Major findings

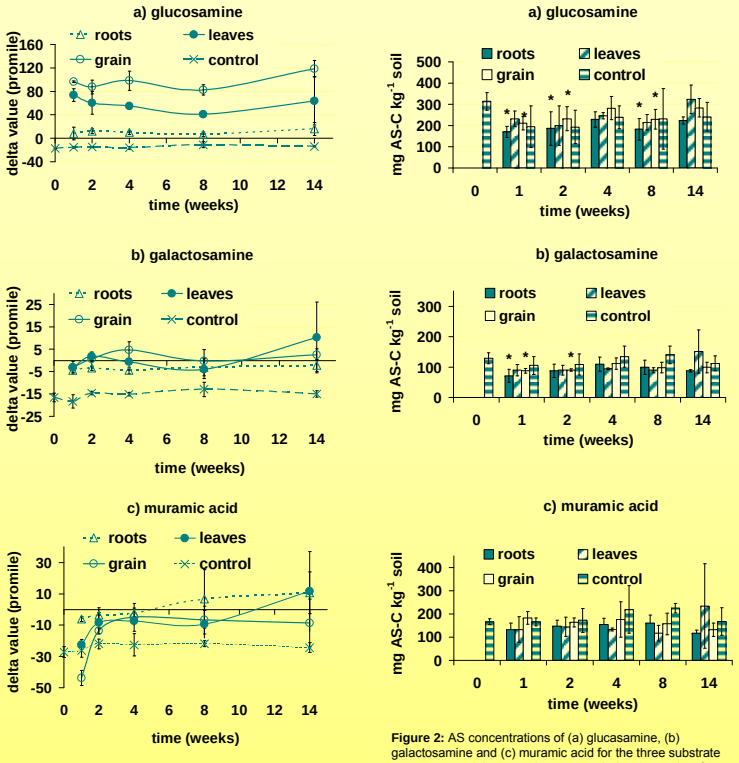


Figure 1: delta ^{13}C values of (a) glucasamine, (b) galactosamine and (c) muramic acid for the three substrate treatments and the control treatment during 14 weeks of incubation

Figure 2: AS concentrations of (a) glucasamine, (b) galactosamine and (c) muramic acid for the three substrate treatments and the control treatment during 14 weeks of incubation. Bars with an asterisk are significantly different ($p<0.05$) from the concentration at time 0 (non incubated soil)

Table 1: Injected carbon concentration, measured $\delta^{13}C$ value of the derivatized compound and the calculated $\delta^{13}C$ value for the internal standards myo-inositol. The true values represent the known amounts of carbon added, the theoretical $\delta^{13}C$ values of the derivatized compounds and the $\delta^{13}C$ values obtained from EA-IRMS measurements.

	C injected (ng µL^{-1})		$\delta^{13}C_{Der}$ (‰)		With F: $\delta^{13}C_{AS}$ (‰)	
	average	stdev	average	stdev	average	stdev
Roots	145.4	30.7	-34.2	1.7	-9.5	4.3
Leaves	156.3	54.0	-33.9	1.7	-8.5	4.9
Grain	115.0	42.7	-32.8	1.8	-5.6	5.5
Control	112.9	37.5	-35.9	1.6	-15.2	4.6
			Theoretical value (derivatized)		Without F: *Measured EA-IRMS value*	
"True" value	133.2		-20.5		-12.6	7‰ bias

Discussion

The uncorrected $\delta^{13}C$ values of derivatized AS standards measured via GC-c-IRMS are more negative than $\delta^{13}C$ values of the original AS and of the derivatizing reagent (-24.38±0.28‰), indicating a considerable fractionation during derivatization (Table 1).

The high bias (7‰) indicates that GC-c-IRMS is inadequate for AS analysis in natural abundance studies, and that the technique also has a limited applicability in ^{13}C enrichment studies.

The lack of differences in bulk AS concentrations between treatments and over time highlights the necessity of introducing a highly enriched tracer to follow new AS production in short term experiments.

The high background AS concentration also indicates a historical stabilization of AS in this sandy soil. Furthermore, the bacterial AS muramic acid appeared to be more stabilized in the sandy soil studied in this experiment compared to literature observations reported for more clayey soils.

Formation of new AS was initially fast and coincided with the exponential growth phase of the soil micro-organisms.

The apparent constant ^{13}C signal after 1 week incubation suggests stabilization or the continuous recycling of newly formed AS.

AS enrichment corresponded to substrate lability.

Fungal glucosamine enrichment was higher compared to enrichment of other AS for all substrate treatments.

Conclusions

This experiment demonstrated substantial shortcomings of the GC-c-IRMS method for isotopic AS analysis. LC-c-IRMS can be suggested as a possible alternative for AS isotope analysis. Avoidance of derivatization, proven to be an important source of error in this experiment, would be the major advantage of this method. Thanks to the very high enrichment of the amended substrate in this experiment, trends of AS sugar dynamics such as fast formation and stabilization or recycling could still be determined.