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Supplement of

Hydrogen dynamics in soil organic matter as determined by ^{13}C and ^2H labeling experiments

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Supplementary material

S1. Incubation characteristics

The isotopic characteristics of the labeling experiment are summarized in the table S1.

Table S1 : Incubation characteristics and the initial isotopic compositions of the labeling experiments. $^{13}A_m$, A_m and A_w are respectively the ^{13}C and 2H abundance of the added labeled source (molecule or water) and $^{13}A_{tot_i}$, A_{tot_i} are respectively the ^{13}C and 2H abundance of the total soil at the initial conditions (time 0).

Experience	Added molecule	mg	2H_2O	$^{13}A_m$ %			$^{13}A_{tot_i}$ % \pm 0.02			A_m or A_w %			A_{tot_i} % \pm 0.005		
				Cambisol	Podzol	Leptosol	Cambisol	Podzol	Leptosol	Cambisol	Podzol	Leptosol	Cambisol	Podzol	Leptosol
$^{13}C^2H + ^1H_2O$	phenylalanine	22.9	-	6.08	11.08	13.08	1.28	1.29	1.22	2.015	2.015	3.015	0.041	0.052	0.026
	glucose	37.5	-	6.08	11.08	16.08	1.28	1.29	1.25	2.015	2.015	3.015	0.057	0.076	0.033
	glucose	12.5	-	-	11.08	-	-	1.15	-	-	3.015	-	-	0.046	-
	glucose	25	-	-	6.08	-	-	1.15	-	-	2.015	-	-	0.056	-
	isoleucine	27.3	-	6.08	11.08	16.08	1.28	1.29	1.25	2.015	2.015	3.015	0.060	0.080	0.035
	palmitic acid	20	-	6.08	11.08	16.08	1.28	1.29	1.25	2.015	2.015	3.015	0.057	0.076	0.033
$^{13}C^1H + ^2H_2O$	phenylalanine	22.9	yes	6.08	11.08	16.08	1.28	1.29	1.22	0.28	0.46	0.27	0.284	0.470	0.275
	glucose	37.5	yes	6.08	11.08	16.08	1.28	1.29	1.25	0.28	0.46	0.27	0.015	0.015	0.015
	glucose	12.5	yes	-	11.08	-	-	1.15	-	-	0.46	-	-	0.015	-
	glucose	25	yes	-	6.08	-	-	1.15	-	-	0.46	-	-	0.015	-
	isoleucine	27.3	yes	6.08	11.08	16.08	1.28	1.29	1.25	0.28	0.46	0.27	0.015	0.015	0.015
	palmitic acid	20	yes	6.08	11.08	16.08	1.28	1.29	1.25	0.28	0.46	0.27	0.015	0.015	0.015
Experiment 3	-	-	yes	-	-	-	1.08	1.08	1.08	0.28	0.46	0.27	0.015	0.015	0.015
Control	-	-	-	-	-	-	1.08	1.08	1.08	-	-	-	0.015	0.015	0.015

S2. Mass balance calculations

All the variables are summarized in the table S2.

Carbon:

$$C_{\text{tot}} = C_{\text{dfm}} + C_{\text{dfs}} \quad (\text{SI1})$$

$$^{13}\text{A}_{\text{tot}} * C_{\text{tot}} = ^{13}\text{A}_{\text{m}} * C_{\text{dfm}} + ^{13}\text{A}_{\text{tot}_0} * C_{\text{dfs}} \quad (\text{SI2.a})$$

$$^{13}\text{A}_{\text{tot}} * C_{\text{tot}} = ^{13}\text{A}_{\text{m}} * C_{\text{dfm}} + ^{13}\text{A}_{\text{tot}_0} * (C_{\text{tot}} - C_{\text{dfm}}) \quad (\text{SI2.b})$$

from SI1 and SI2 we derive:

$$C_{\text{dfm}} = (^{13}\text{A}_{\text{tot}} - ^{13}\text{A}_{\text{tot}_0}) / (^{13}\text{A}_{\text{m}} - ^{13}\text{A}_{\text{tot}_0}) * C_{\text{tot}} \quad (1)$$

Hydrogen:

After freeze-drying, hydrogen is considered in three compartments: (i) NEH derived from labeled molecule atoms, (ii) NEH from unlabeled sources, and (iii) exchangeable hydrogen, which is by definition equilibrated with the atmosphere with a given fractionation ε_1

$$H_{\text{tot}} = H_{\text{dfm}} + H_{\text{dfs}} + H_{\text{e}} \quad (\text{SI3})$$

$$A_{\text{tot}} * H_{\text{tot}} = A_{\text{m}} * H_{\text{dfm}} + A_{\text{dfs}} * H_{\text{dfs}} + (A_{\text{atm}} + \varepsilon_1) * H_{\text{e}} \quad (\text{SI4})$$

from SI3 and SI4 we derive:

$$A_{\text{tot}} * H_{\text{tot}} = A_{\text{m}} * H_{\text{dfm}} + [A_{\text{dfs}} * H_{\text{dfs}} + (A_{\text{atm}} + \varepsilon_1) * H_{\text{e}}] / (H_{\text{dfs}} + H_{\text{e}}) * (H_{\text{tot}} - H_{\text{dfm}}) \quad (\text{SI5})$$

The term $[A_{\text{dfs}} * H_{\text{dfs}} + (A_{\text{atm}} + \varepsilon_1) * H_{\text{e}}] / (H_{\text{dfs}} + H_{\text{e}})$ is the average composition of unlabeled hydrogen. It is derived from the analysis of the unlabeled sample:

$$H_{\text{tot}_0} = H_{\text{dfs}} + H_{\text{e}} \quad (\text{SI6})$$

$$A_{\text{tot}_0} = [A_{\text{dfs}} * H_{\text{dfs}} + (A_{\text{atm}} + \varepsilon_1) * H_{\text{e}}] / (H_{\text{dfs}} + H_{\text{e}}) \quad (\text{SI7})$$

from SI5 and SI7 we derive:

$$A_{\text{tot}} * H_{\text{tot}} = A_{\text{m}} * H_{\text{dfm}} + A_{\text{tot}_0} * (H_{\text{tot}} - H_{\text{dfm}}) \quad (\text{SI8})$$

$$H_{\text{dfm}} = [(A_{\text{tot}} - A_{\text{tot}_0}) / (A_{\text{m}} - A_{\text{tot}_0})] * H_{\text{tot}} \quad (2)$$

By similarity, for Experiment 2 (labeled water), we write:

$$H_{dfw} = (A_{tot} - A_{tot_0}) / (A_w - A_{tot_0}) * H_{tot} \quad (3)$$

These equations are based on approximations: the isotope fractionation term between atmosphere and exchangeable H is supposed to be equal in labeled and unlabeled samples: this is supported by the fact that the amount of exchangeable hydrogen is considered unaffected by the (small) amount of introduced labeled material. The amount of unlabeled HNE (H_{dfs}) and its abundance (A_{dfs}) are considered equal in the labeled and unlabeled samples. Inequality would affect the denominator in equations (2) and (3). Equality is a numerical approximation without consequence, owing to the very high enrichment of labeled material (A_m , A_w , see table S1).

Table S2: Definition of the variables used for mass balance calculation

	Variables	Definition
Quantity (mg g ⁻¹ dry soil)	C_{tot}	Total amount of carbon in the soil
	H_{tot}	Total amount of hydrogen in the dry soil
	C_m	Initial amount of carbon in the added molecule
	H_m	Initial amount of non-exchangeable hydrogen in the added molecule
	H_w	Total amount of water hydrogen in the soil
	H_e	Amount of exchangeable hydrogen in the dry soil
	C_{dfs}	Amount of unlabeled carbon already present in the soil
	C_{dfm}	Amount of carbon derived from molecule
	H_{dfs}	Amount of unlabeled non-exchangeable hydrogen present in the soil
	H_{dfw}	Amount of non-exchangeable hydrogen derived from water
	H_{dfm}	Amount of non-exchangeable hydrogen derived from molecule
	Abundance	$^{13}A_{tot}$
A_{tot}		2H abundance of the total bulk soil
$^{13}A_{tot_0}$		^{13}C abundance of the unlabeled experiment (control)
A_{tot_0}		2H abundance of the unlabeled experiment (control)
$^{13}A_m$		Initial ^{13}C abundance of the labeled molecule
A_m		Initial 2H abundance of the labeled molecule
A_w		Initial 2H abundance of the labeled water
A_{atm}		2H abundance of the atmosphere

To calculate the incorporation of the water hydrogen coming from the mineralisation of the added molecule (recycling), we assume that the labelled molecule is completely mineralised in water. The resulting isotopic composition of water in experiment 1 (A_{w2}) can be calculated from the isotopic composition of the labelled molecule as follow:

$$A_{w2} = (A_m * H_m) / H_w \quad (S19)$$

Then, the amount of non-exchangeable hydrogen that can be derived from this water (H_{dfw2}) can be calculated using the value H_{dfw} calculated in equation (3) :

$$H_{dfw2} = (A_{w2} - A_{tot_0}) / (A_m - A_{tot_0}) * H_{dfw} \quad (4)$$

The proportion of deuterium derived from the molecule but incorporated in the soil by the water is given by $(H_{dfw2}/H_{dfm}) * 100$ where H_{dfm} is calculated in equation (2).

S3. Propagation error calculation

Uncertainties on the element and isotope ratio measurements affect the estimate of the amount of labeled-source derived carbon or hydrogen atoms. To assess the uncertainty $\sigma_{C_{dfm}}$ (err7), $\sigma_{H_{dfm}}$ (err8), $\sigma_{H_{dfw}}$ (err9) or on the calculated values C_{dfm} (Eq. 6), H_{dfm} (Eq. 7), and H_{dfw} (Eq.8) we calculated the statistical error propagation of the uncertainties on measured isotopic compositions and element content of replicated samples presenting in supporting information.

$$\sigma_{C_{dfm}}^2 = (\sigma_{^{13}A_{tot}}^2 + \sigma_{^{13}A_{tot_0}}^2) * [C_{tot} / (A_m - A_{tot_0})]^2 + (\sigma_{^{13}A_m}^2 - \sigma_{^{13}A_{tot_0}}^2) * (A_{tot} - A_{tot_0})^2 * C_{tot}^2 / (A_m - A_{tot_0})^2 + \sigma_{C_{tot}}^2 * [(A_{tot} - A_{tot_0}) / (A_m - A_{tot_0})]^2 \quad (err6)$$

$$\sigma_{H_{dfm}}^2 = (\sigma_{A_{tot}}^2 + \sigma_{A_{tot_0}}^2) * [H_{tot} / (A_m - A_{tot_0})]^2 + (\sigma_{A_m}^2 - \sigma_{A_{tot_0}}^2) * (A_{tot} - A_{tot_0})^2 * H_{tot}^2 / (A_m - A_{tot_0})^2 + \sigma_{H_{tot}}^2 * [(A_{tot} - A_{tot_0}) / (A_m - A_{tot_0})]^2 \quad (err7)$$

$$\sigma_{H_{dfw}}^2 = (\sigma_{A_{tot}}^2 + \sigma_{A_{tot_0}}^2) * [H_{tot} / (A_w - A_{w_0})]^2 + (\sigma_{A_w}^2 - \sigma_{A_{w_0}}^2) * (A_{tot} - A_{tot_0})^2 * H_{tot}^2 / (A_w - A_{w_0})^2 + \sigma_{H_{tot}}^2 * [(A_{tot} - A_{tot_0}) / (A_w - A_{w_0})]^2 \quad (err8)$$

where the indices of the terms σ_x stand for the standard deviation of the respective variable. In this calculation, the uncertainties $\sigma_{A_m}^2$, $\sigma_{A_w}^2$ and $\sigma_{^{13}A_m}^2$ were considered as negligible.

S4. $\delta^{13}\text{C}$ and $\delta^2\text{H}$ results of the incubation samples

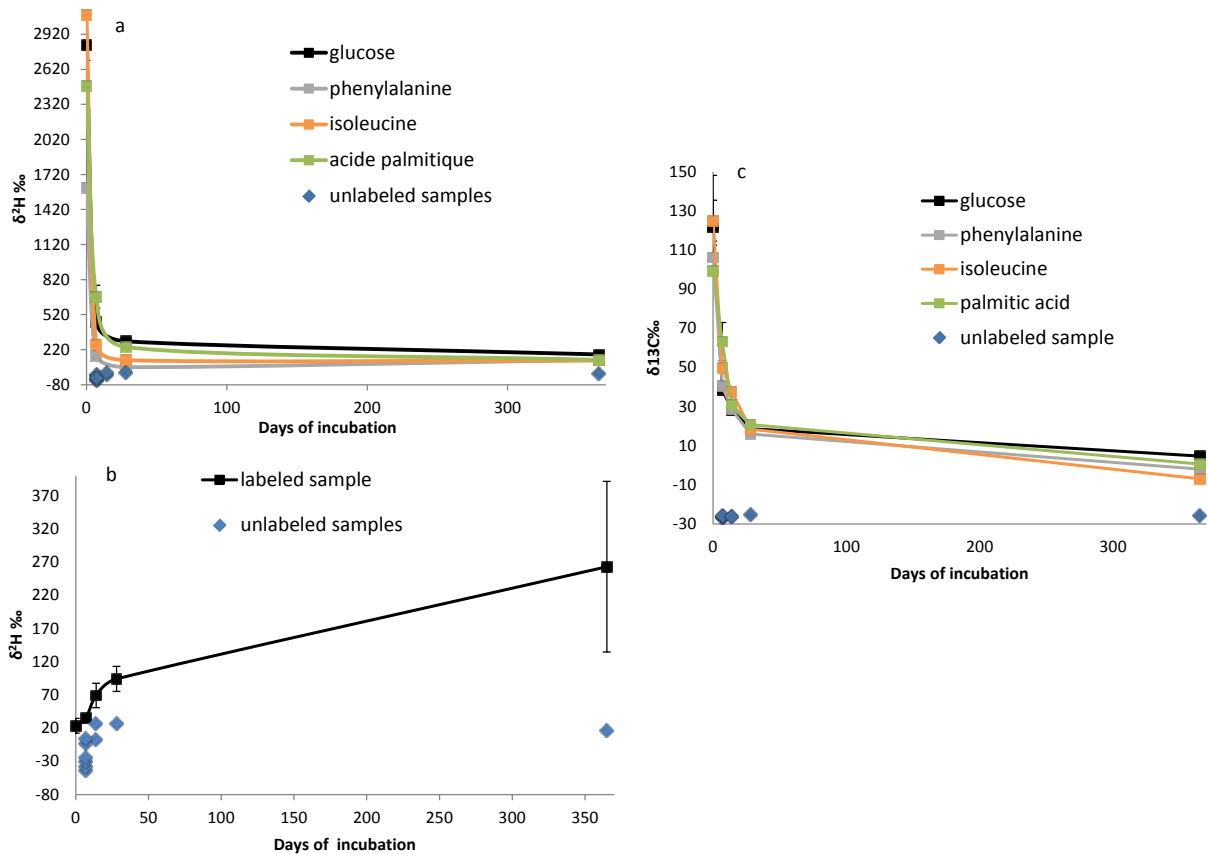


Figure S4.1: Cambisol ^{13}C and ^2H isotopic variation a. $\delta^2\text{H}$ variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. b. $\delta^2\text{H}$ variation through time of bulk soil that received labeled water and unlabeled samples. c. $\delta^{13}\text{C}$ variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. Standard deviations are less than 3 ‰ for unlabeled samples.

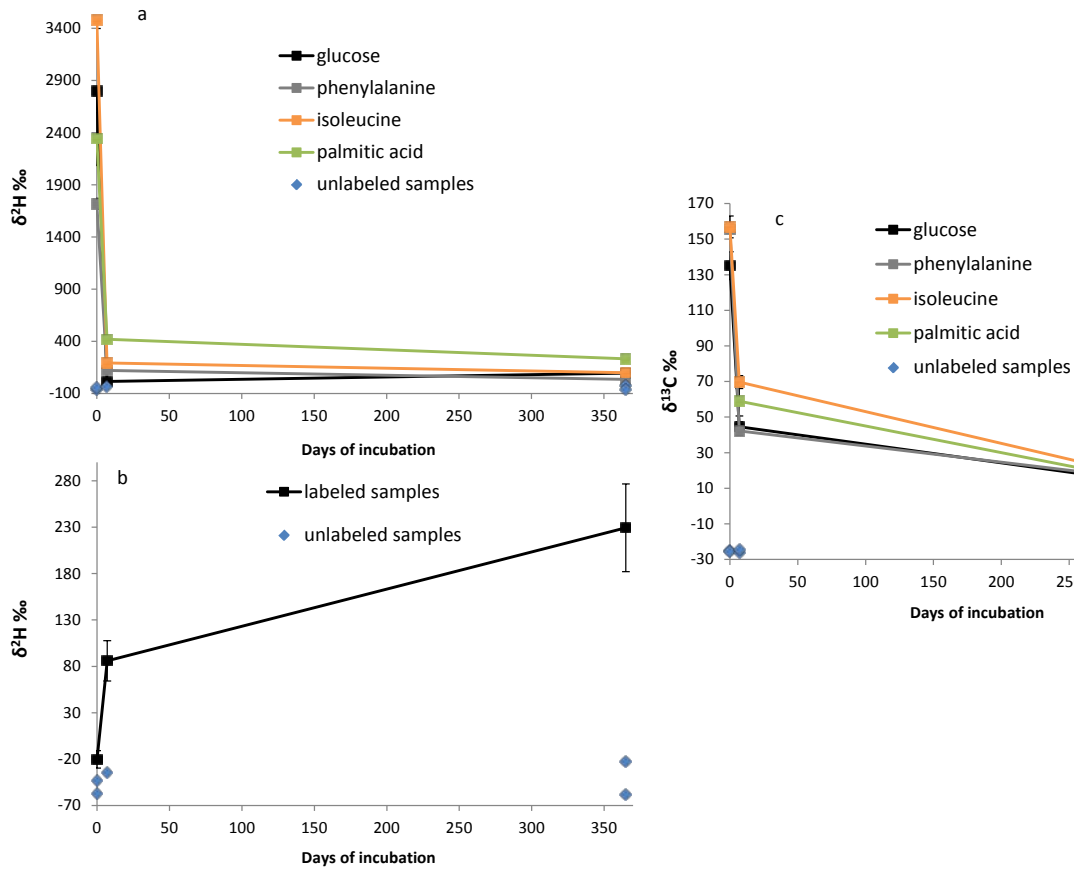


Figure S4.2: Podzol ^{13}C and ^2H isotopic variation a. $\delta^2\text{H}$ variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. b. $\delta^2\text{H}$ variation through time of bulk soil that received labeled water and unlabeled samples. c. $\delta^{13}\text{C}$ variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. Standard deviations are less than 3 ‰ for unlabeled samples.

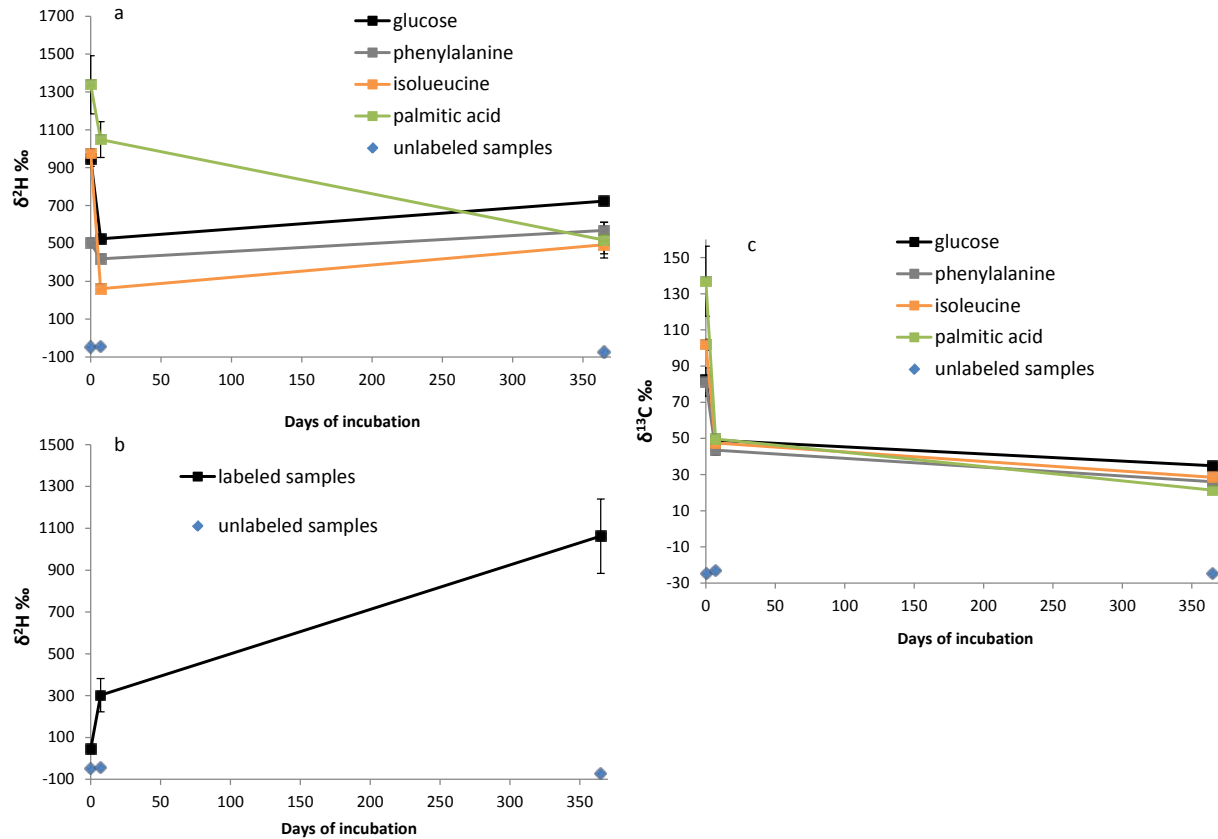


Figure S4.3: Leptosol ¹³C and ²H isotopic variation a. δ²H variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. b. δ²H variation through time of bulk soil that received labeled water and unlabeled samples. c. δ¹³C variation through time of the bulk soil that received labeled glucose, phenylalanine, isoleucine and palmitic acid and unlabeled samples. Standard deviations are less than 3 ‰ for unlabeled samples.