Flux analysis of free amino sugars and amino acids in soils by isotope tracing with a novel liquid chromatography-high resolution mass spectrometry platform

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Supplementary Contents

Soil sampling procedure

Soil was collected from a mixed spruce-beech forest in Gumpenstein, Austria (47°50'N, 14°13'E; 768 m elevation) and from an arable field in Moarhof, Austria (47°51'N, 14°06'E; 70 8m elevation). Sieved (<2 mm) soils were stored at 15 °C incubator after return to laboratory.

Table S1: Selected properties of the soil samples

	Forest GF	Arable MA
Depth (cm)	0-15	0-15
Bedrock	Silicate	Limestone
Dominant vegetation	Spruce, mosses, Vaccinium myrtillus	Barley
pH (H_2O)	4.1	8.2
Total Organic C, mg g^{-1}	49.9	47.0
Total N, mg kg^{-1}	2.54	4.77
Organic P, mg kg^{-1}	0.31	0.72
Cation exchange capacity, cmolc kg^{-1}	9.75	33.6
Base saturation, %	5.2	99.9

Table S2: Purity of isolated peptidoglycan and isotope labeling efficiency in the hydrolysates of ^{15}N and ^{13}C labeled peptidoglycan

Briefly, cells were harvested by centrifugation (14,000 g, 15 min) at 4 °C when OD_{600} reached around 1.0. Aliquots of 10 mg fresh cell mass were washed with 1 x TBS and boiled in 2 mL 10% (w/v) trichloroacetic acid solution for 30 min. Insoluble materials were collected by centrifugation (14000 g, 10 min) and treated with 2 mL hot 5% (w/v) sodium docecyl sulfate at 100 °C for 30 min. Pellets were washed with water 5 times at room temperature for complete removal of detergent. Pellets were incubated with 2 mL trypsin (100 μg mL^{-1}) overnight at 37 °C and subsequently boiled for 15 min to inactivate the enzyme. Insoluble materials were then washed with water 3 times. Finally, raw peptidoglycan was collected by centrifugation at 14000 g for 10 min.

	Purity of isolated peptidoglycan	^{15}N tracer	^{13}C internal standard
metabolite	molar ratio to glucosamine	atom%^{15}N (%)	atom%^{13}C (%)
glucosamine	1.00	99.9	99.9
muramic acid	0.81	99.9	99.9
mDAP	0.70	99.9	99.9
alanine	1.40	99.9	99.9
glutamic acid	1.26	95.0	96.1
glycine	0.18	99.9	99.9
lysine	0.16	79.4	81.3
leucine	0.12	68.5	58
serine	0.04	99.9	68.5
histidine	0.06	73.3	78.0

All other amino acids (valine, phenylalanine, cysteine, methionine, etc.) were below the LOD.

Table S3: Concentrations of amino sugars and amino acids in the ^{15}N tracer mix and the ^{13}C internal standards

	Concentration of ^{13}C metabolites in the internal standard (μM)	Concentration of ^{15}N metabolites in the tracer solution (μM)
glucosamine	1.379	0.655
muramic acid	1.042	0.569
mDAP	1.917	0.848
alanine	6.617	2.818
glutamic acid	6.471	3.543
glycine	3.847	1.577
lysine	2.938	1.703
valine	1.806	0.756
leucine	0.967	0.567
methionine	0.378	0.002
phenylalanine	0.670	0.399
proline	1.619	0.664
serine	2.340	0.717
threonine	1.783	0.884
tyrosine	0.624	0.375
asparagine	0.006	0.002
aspartic acid	1.931	0.985
glutamine	0.001	0.001
arginine	1.253	0.793
histidine	0.691	0.478
L-alanine	5.481	2.131
D-alanine	1.232	0.786
L-glutamic acid	4.823	2.605
D-glutamic acid	1.874	0.988

Table S4: Isotope calibration for amino compounds with LC/MS

Compound	Slope	y-Intercept	Concentration dependence	R^2	p-value
lysine	0.89	-0.003	n.s.	0.9786	<0.0001
glutamic acid	1.02	-0.006	n.s.	0.9998	<0.0001
aspartic acid	1.02	-0.002	n.s.	0.9967	<0.0001
methionine	1.05	-0.002	n.s.	0.9995	<0.0001
proline	0.99	-0.004	n.s.	0.9998	<0.0001
serine	1.04	-0.007	n.s.	0.9996	<0.0001
threonine	0.91	0.061	n.s.	0.9754	<0.0001
leucine	1.03	-0.001	n.s.	0.9996	<0.0001
arginine	0.99	-0.012	n.s.	0.9935	<0.0001
alanine	1.01	0.009	n.s.	0.9722	<0.0001
valine	1.01	-0.011	n.s.	0.9829	<0.0001
histidine	0.92	0.002	n.s.	0.9945	<0.0001
tyrosine	0.86	0.016	n.s.	0.9959	<0.0001
phenylalanine	0.95	0.012	n.s.	0.9975	<0.0001
glycine	1.01	0.013	n.s.	0.9878	<0.0001

Multiple regression analysis was performed between percentage of ^{15}N peak area (^{15}N peak area/(^{15}N peak area+^{14}N peak area)*100) against ^{15}N atom percentage and compound concentration of external standards.

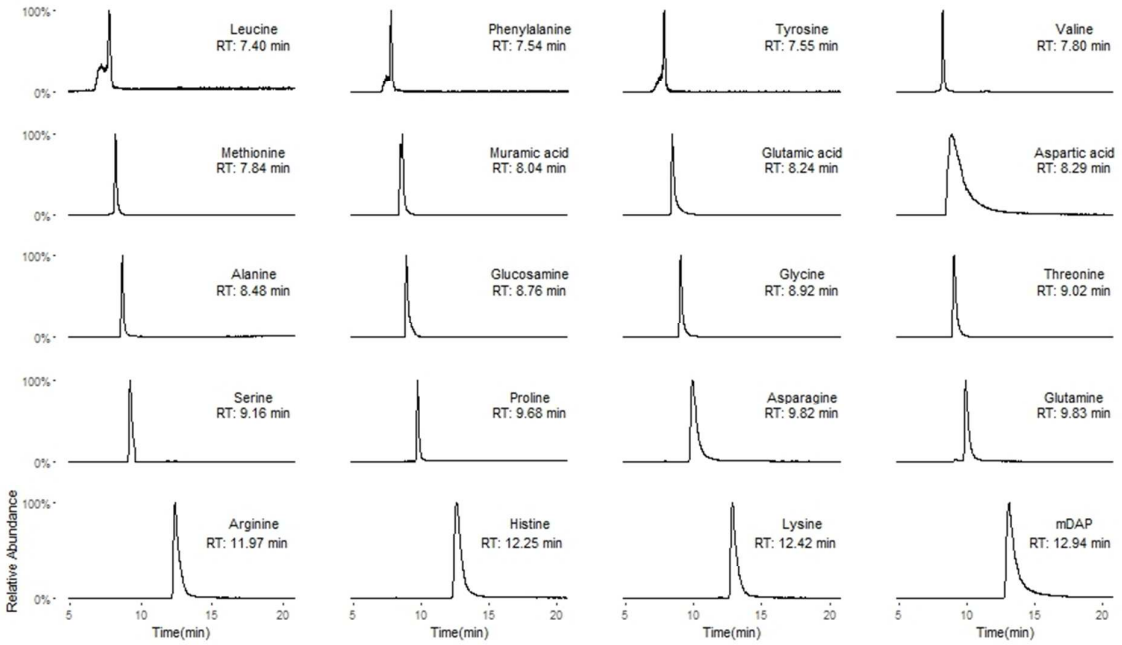
Table S5: Concentrations of free amino sugars and amino acids of a forest and an arable soil, and their gross influx and efflux rates and mean residence times

	Free Pool ($\mu g\ N\ g^{-1}$ d.w.)		Influx ($\mu g\ N\ g^{-1}$ d.w. d^{-1})		Efflux ($\mu g\ N\ g^{-1}$ d.w. d^{-1})		Mean residence time (hours)	
Soil type	Forest	Arable	Forest	Arable	Forest	Arable	Forest	Arable
glucosamine	0.791±0.286	0.575±0.184	0.564±0.118	0.817±0.354	0.187±0.019	0.283±0.131	2.11	1.05
muramic acid	0.003±0.002	0.009±0.003	0.1e-4±0. 5e6	0.008±0.001	0.023±0.003	0.036±0.011	18.71	0.41
mDAP	0.113±0.046	0.067±0.011	0.242±0.002	0.195±0.002	0.161±0.001	0.167±0.002	0.56	0.37
alanine	1.397±0.043	1.861±0.19	0.984±0.025	1.543±0.236	0.592±0.065	1.173±0.39	1.77	1.37
glutamic acid	2.392±0.28	3.192±0.044	1.752±0.186	0.995±0.268	1.391±0.016	0.83±0.001	1.52	3.5
glycine	1.407±0.182	0.952±0.037	1.207±0.133	1.388±0.385	0.427±0.015	1.162±0.03	1.72	0.75
lysine	0.77±0.001	1.48±0.018	0.328±0.006	0.652±0.044	0.573±0.007	0.5±0.016	1.71	2.57
valine	0.471±0.088	0.83±0.056	0.309±0.045	0.249±0.232	0.138±0.009	0.259±0.008	2.11	3.27
leucine	0.174±0.034	0.537±0.009	0.149±0.02	0.266±0.104	0.107±0.002	0.263±0.032	1.36	2.03
methionine	0.012±0.001	0.093±0.001	0.006±0.001	0.095±0.018	0.006±0.001	0.092±0.002	2	0.99
phenylalanine	0.143±0.031	0.29±0.007	0.142±0.024	0.224±0.027	0.105±0.003	0.217±0.013	1.16	1.32
proline	0.189±0.035	0.152±0.007	0.18±0.025	0.112±0.019	0.129±0.006	0.08±0.016	1.22	1.58
serine	0.897±0.126	0.846±0.162	0.858±0.092	1.376±0.88	0.276±0.075	1.114±0.837	1.58	0.68
threonine	0.498±0.08	0.549±0.01	0.478±0.068	0.362±0.018	0.273±0.004	0.357±0.055	1.33	1.53
tyrosine	0.103±0.013	0.153±0.001	0.124±0.011	0.198±0.009	0.106±0.001	0.194±0.005	0.9	0.78
asparagine	0.049±0.037	0.041±0.057	0.135±0.029	0.046±0.002	0.105±0.012	0.027±0.006	0.41	1.12
aspartic acid	2.815±2.649	1.632±0.665	4.775±0.08	4.511±0.132	0.931±0.022	2.627±0.037	0.99	0.46
glutamine	0.017±0.001	0.009±0.002	0.046±0.005	0.014±0.001	0.046±0.011	0.013±0.003	0.37	0.67
arginine	2.141±0.152	0.454±0.025	2.199±0.154	0.298±0.043	2.104±0.271	0.176±0.005	1	1.92
histidine	0.423±0.051	0.153±0.007	0.477±0.055	0.43±0.067	0.403±0.021	0.389±0.034	0.96	0.37
L-alanine	0.945±0.067	1.629±0.449	0.746±0.208	0.812±0.173	0.488±0.028	0.472±0.009	1.53	2.54
D-alanine	0.478±0.009	0.452±0.049	0.072±0.008	0.426±0.077	0.06±0.009	0.431±0.113	7.24	1.05
L-glutamic acid	2.536±0.638	2.595±0.419	1.548±0.229	0.449±0.181	0.928±0.029	0.727±0.185	2.05	4.41
D-glutamic acid	0.116±0.007	0.744±0.22	0.402±0.011	0.375±0.08	0.266±0.073	0.069±0.017	0.35	3.35

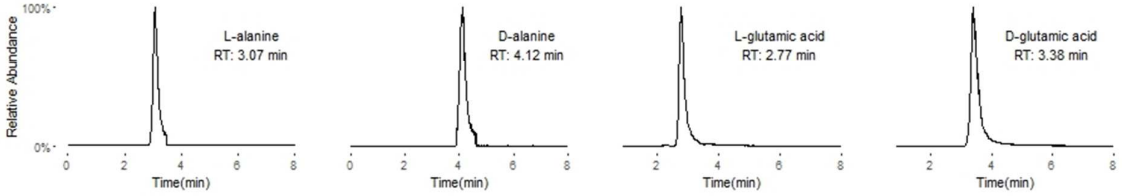
Free pools, influxes and effluxes are represented as means±SD. Fluxes of cysteine and tryptophan are not listed in this table, as the concentrations of these two amino acids in labeled (^{15}N and ^{13}C) algal amino acid mixtures were below limit of quantification.

Figure S1: Extracted ion chromatograms (EIC) of amino compounds (1µM) after separation by HILIC chromatography/chiral chromatography and detection by Orbitrap-MS

A: HILIC separation



B: Chiral separation



Remark: leucine and isoleucine, as well as glucosamine, mannosamine, and galactosamine could not be separated by HILIC chromatography

Figure S2: Mass spectrometric separation of isobaric ^{15}N-glycine and $^{13}C_1$-glycine by Orbitrap-MS at a resolution of 50,000

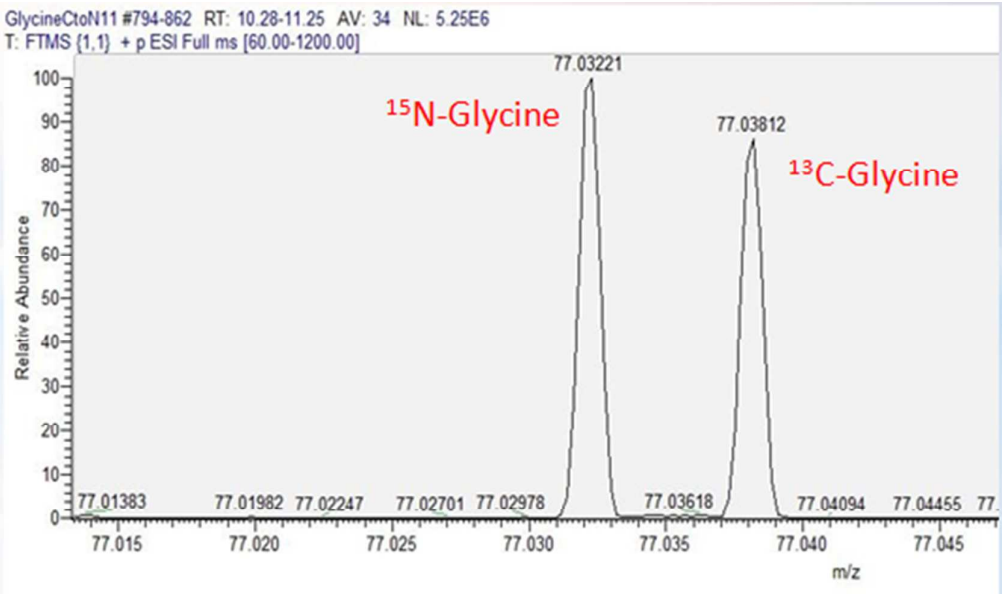
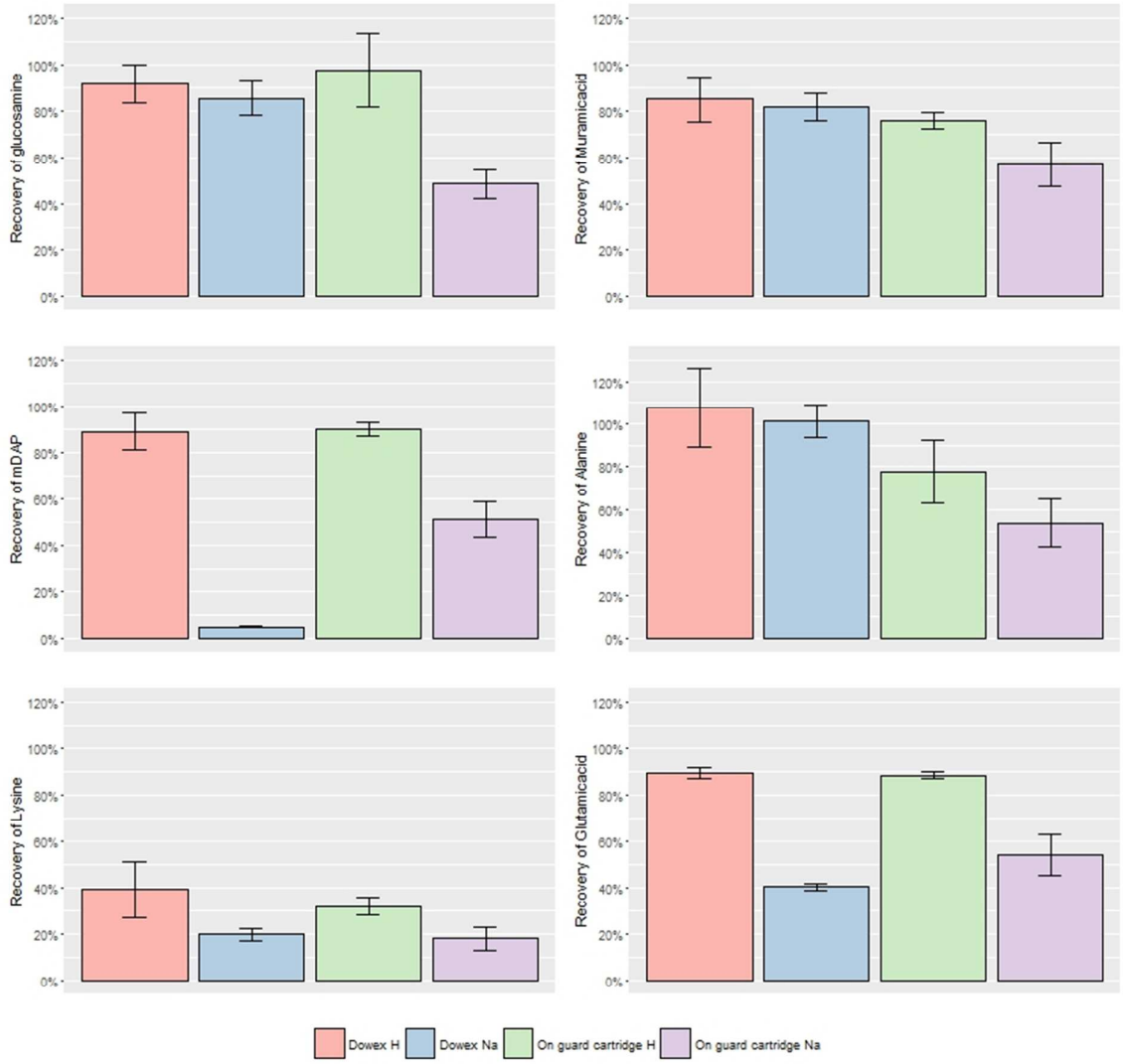


Figure S3: Recovery of selected metabolites on different cation-exchange resins



Values are means ± SD (n=3). On guard cartridges (H^+ form or Na^+ form) were obtained from Thermo Fisher Scientific (Bremen, Germany).