microplastics

MDPI

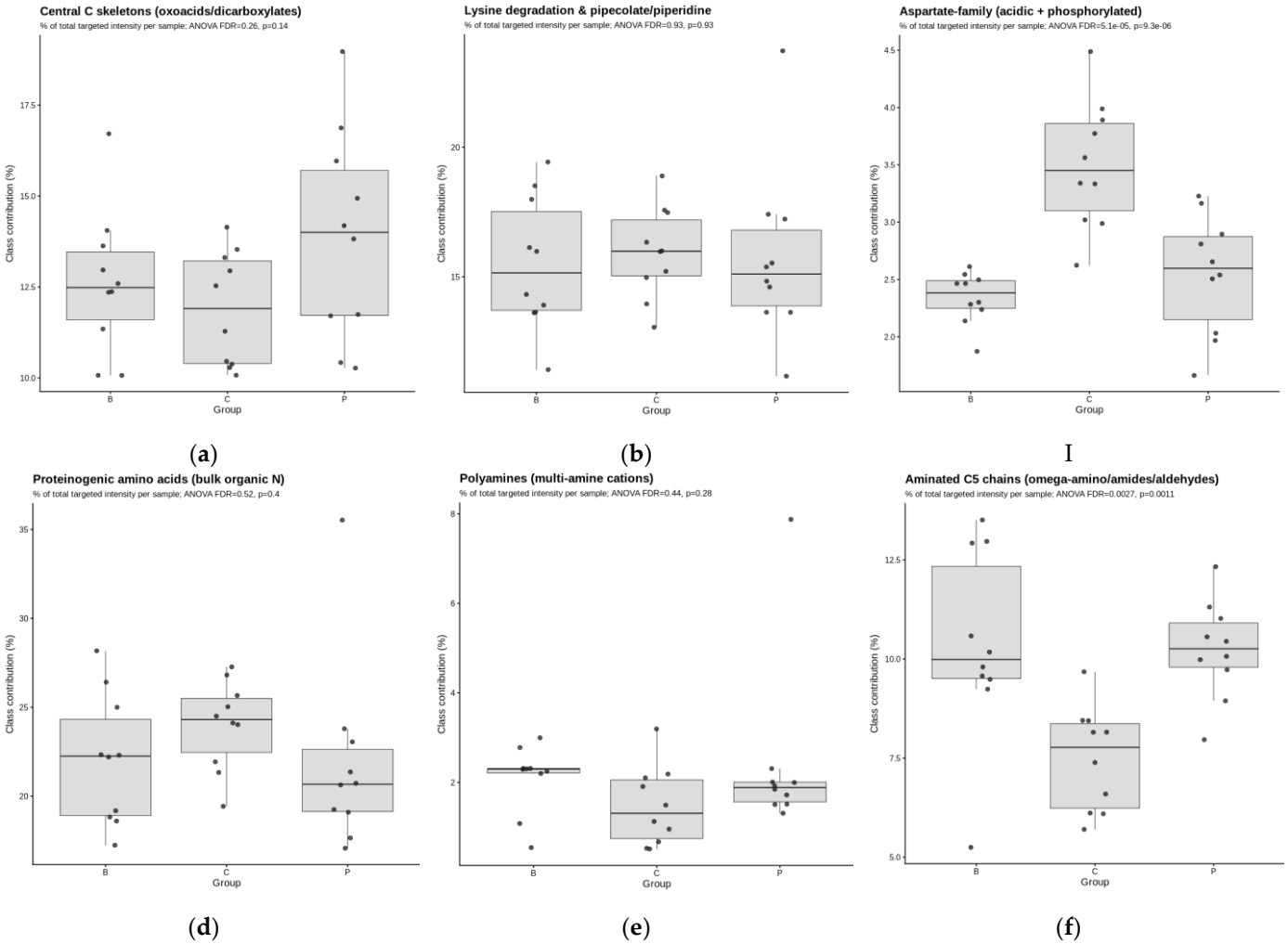
Article

Plastic and Biodegradable Mulch Reshapes the Nitrogen Cycling Process in Soil

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Supplementary material Figure S1



(**a**) (**b**) I

(**d**) (**e**) (**f**)

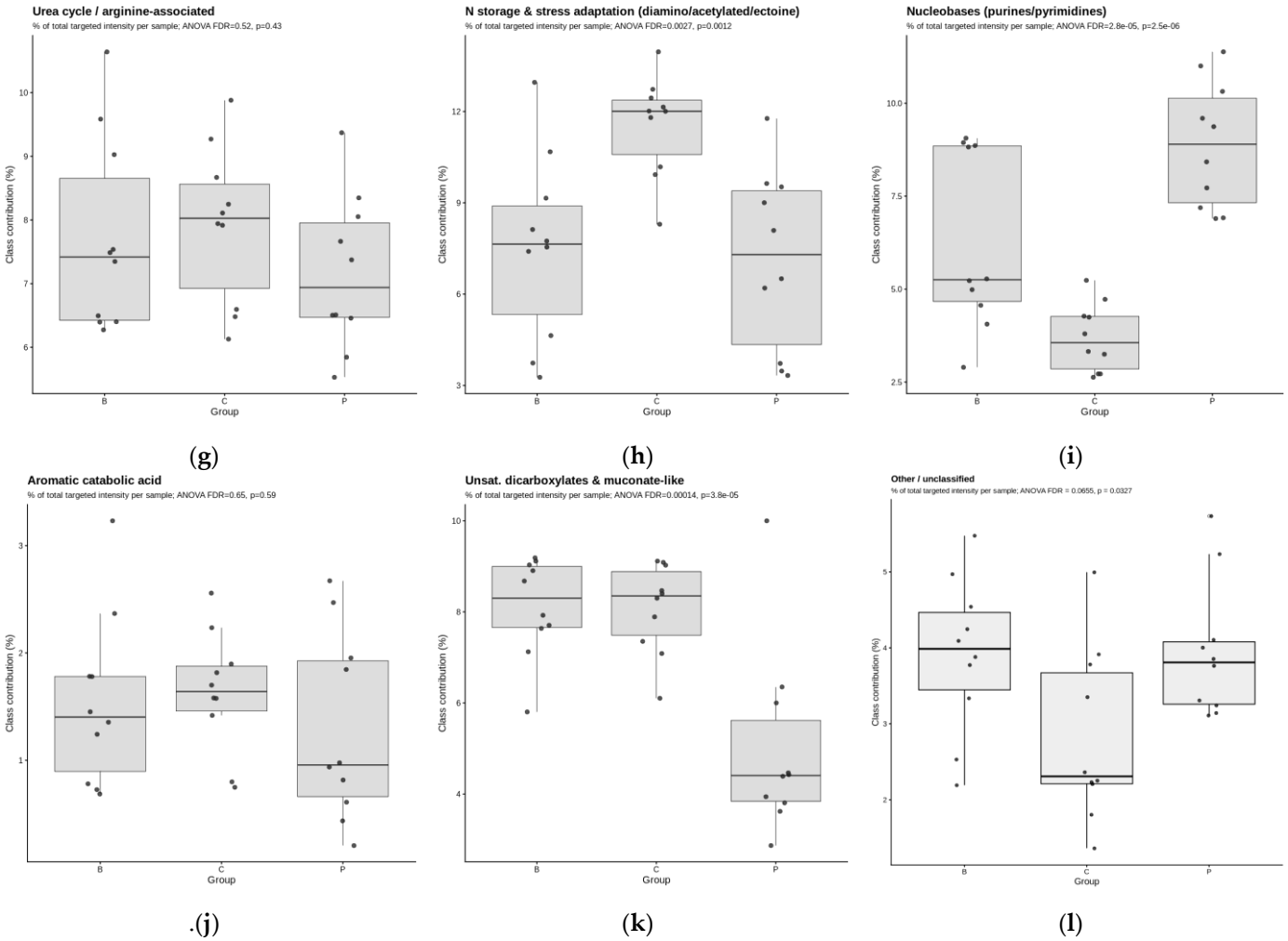
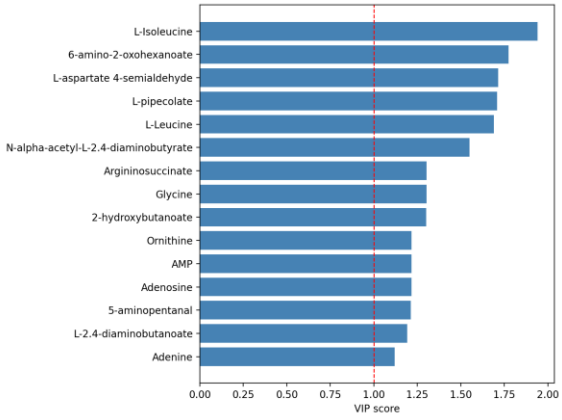
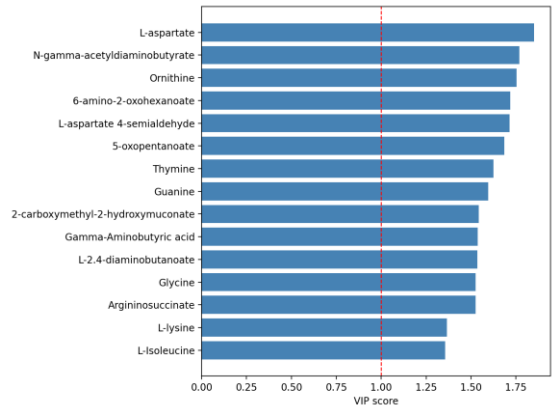


Figure S1. Groups of identified metabolites classes. (**a**) central C skeletons (oxoacids/dicarboxylates); (**b**) lysine degradation and pipecolate/piperidine; (**c**) aspartate-family (acidic + phosphorylated); (**d**) proteinogenic amino acids (bulk organic N); (**e**) polyamines (multi-amine cations); (**f**) aminated C5 chains (omega-amino/amides/aldehydes); (**g**) urea cycle/arginine-associated; (**h**) N storage and stress adaptation (diamino/acetylated/ectoine; (**i**) nucleosides (purines/pyrimidines); (**j**) aromatic catabolic acid; (**k**) unsaturated dicarboxylates and muconate-like; (**l**) other/unclassified.

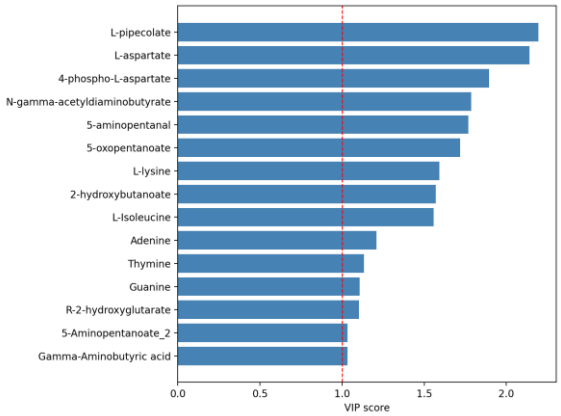
Supplementary material Figure S2



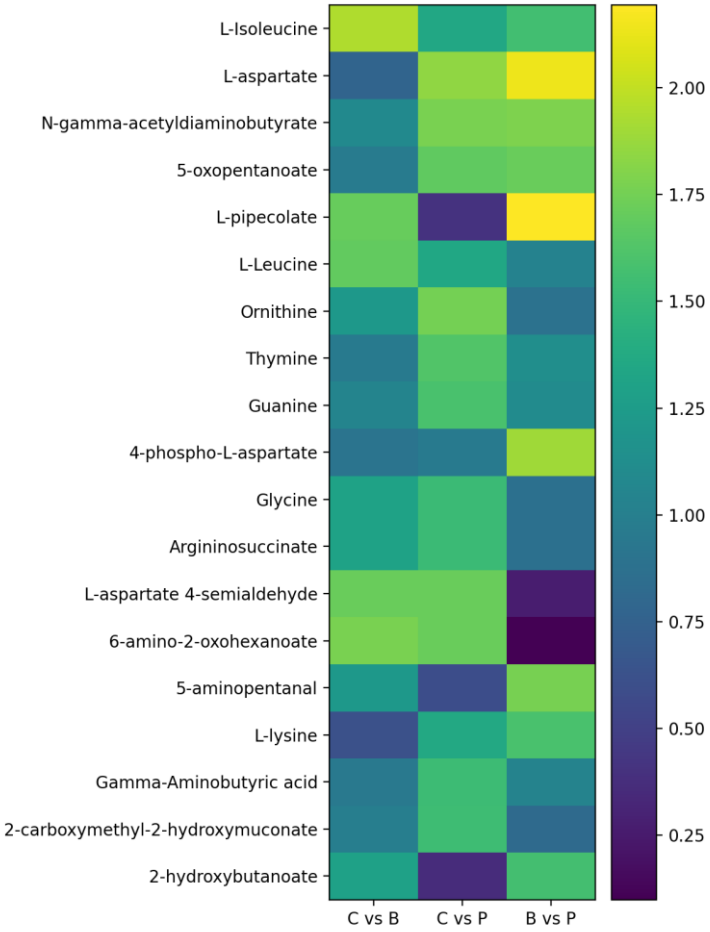
(**a**)



(**b**)



(**c**)



(**d**)

Figure S2. VIP analysis of OPLS-DA models: (**a**) top 15 metabolites by VIP for the OPLS-DA model contrasting C *vs*. B; (**b**) top 15 metabolites by VIP for the OPLS-DA model contrasting C *vs*. P; **c**) top 15 metabolites by VIP for the OPLS-DA model contrasting P *vs*. B; **d**) VIP heatmap across pairwise comparisons.

Supplementary material Table S1.

Table S1. Pathways related to the N cycle.

Pathway	N-cycle relevance
Transamination	Transfer of amino group (-NH_2) between amino acids and keto-acids; central to N assimilation
Reductive amination	Incorporates inorganic N () into organic molecules (e.g., glutamate synthesis by GDH)
Oxidative deamination	Release from amino acids back to the N pool
Biosynthesis	Amino acids biosynthesis involves N incorporation from glutamine/glutamate
Biosynthesis (Aspartate family)	Aspartate is a key N-carrier; aspartate family biosynthesis is core to N metabolism
Biosynthesis (DABA)	Diaminobutyric acid biosynthesis – N-rich compound, linked to osmoprotectant/N storage
Osmoprotectant synthesis	Ectoine/DABA synthesis involves N-rich compounds, relevant to N cycling under stress
Degradation	Amino acid degradation releases N back to the cycle
Lysine degradation	Lysine is N-rich (two amino groups); its degradation releases N
Lysine degradation to TCA	Same as above, channeling N-containing intermediates into TCA
Degradation (Pipecolate)	Pipecolate pathway of lysine degradation — N-containing ring compound
Degradation (Aminoadipate)	Aminoadipate is a lysine degradation intermediate carrying an amino group
Racemization	Interconversion of amino acid stereoisomers — relevant to N compound availability
Decarboxyation	Amino acid decarboxylation produces amines — part of N transformation
TCA cycle	Provides carbon skeletons (2-oxoglutarate, oxaloacetate) that accept in N assimilation

Supplementary material Table S2

Table S2. List of CBM network reactions.

No.	R	Reaction summary	Pathway	N-cycle related
1.	R1a	Succinate → Fumarate	TCA cycle	Yes
2.	R1b	Fumarate → Malate	TCA cycle	Yes
3.	R1c	Malate → Oxaloacetate	TCA cycle	Yes
4.	R1d	Oxaloacetate → 2-Oxoglutarate	TCA cycle	Yes
5.	R41	2-Oxoglutarate → Succinate	TCA cycle	Yes
6.	R2	2-Oxoglutarate → 2-Hydroxyglutarate	Reductive	No
7.	R3	2-Hydroxyglutarate → Glutarate	Oxidative	No
8.	M1	Glutarate → Glutaryl-CoA	CoA activation	No
9.	R4	Glutaryl-CoA → 5-Oxopentanoate	CoA dependent	No
10.	R5	5-Oxopentanoate → 5-Aminopentanoate	Transamination	Yes
11.	M5	5-Aminopentanoate → Glutarate	Lysine degradation	Yes
12.	R6a	2-Oxoglutarate + Acetyl-CoA → Homocitrate	Biosynthesis	Yes
13.	M6	Homocitrate → Homoisocitrate	Biosynthesis	Yes
14.	M7	Homoisocitrate → 2-Oxoadipate	Biosynthesis	Yes
15.	R6b	2-Oxoadipate → Lysine	Biosynthesis	Yes
16.	R7	Lysine → 5-Aminopentanoate	Degradation	Yes
17.	R8	5-Aminopentanoate → 5-Aminopentanal	Oxidation/ Transamination	No
18.	R9	Lysine → Cadaverine	Decarboxylation	Yes
19.	R10	5-Aminopentanal → Cadaverine	Reductive deamination	Yes
20.	R11	Cadaverine → 5-Aminopentanoate	Oxidative deamination	Yes
21.	R12	L-Lysine → D-Lysine	Racemization	Yes
22.	R13	L-Lysine → delta-1-Piperideine-2-carboxylate	Degradation (Pipecolate)	Yes
23.	R14	delta-1-Piperideine-2-carboxylate → L-Pipecolate	Degradation (Pipecolate)	Yes
24.	R15	L-Pipecolate → S-2,3,4,5-Tetrahydropyridine-2-carboxylate	Degradation (Pipecolate)	Yes
25.	R16	S-2,3,4,5-Tetrahydropyridine-2-carboxylate → L-2-Amino-adipate-6-semialdehyde	Degradation (Pipecolate)	Yes
26.	R17	L-2-Aminoadipate-6-semialdehyde → L-2-Aminoadipate	Degradation (Aminoadipate)	Yes
27.	R18	L-2-Aminoadipate → 2-Oxoadipate	Degradation (Aminoadipate)	
28.	R19	2-Oxoadipate → Glutaryl-CoA to 2-Oxoglutarate	Degradation of TCA	No
29.	R20	L-Lysine → 6-Amino-2-oxohexanoate	Alternative degradation	No
30.	R21	6-Amino-2-oxohexanoate → L-2-Aminoadipate-6-semialde-hyde	Alternative degradation	No
31.	R22	L-Aspartate → 2-Phospho-L-aspartate	Biosynthesis (Aspartate family)	Yes
32.	R23	2-Phospho-L-aspartate → L-aspartate-4-smialdehyde	Biosynthesis (Aspartate family)	Yes
33.	R24	L-aspartate-4-smialdehyde → L-2.4-diaminobutanoate	Biosynthesis (DABA)	Yes
34.	R25	L-2.4-diaminobutanoate → N-gamma-Acetyl-diaminobutyr-ate	Osmoprotectant synthesis	Yes
35.	R26	N-gamma-Acetyl-diaminobutyrate → Ectoine	Osmoprotectant synthesis	Yes
36.	R27	L-2,4-Diaminobutanoate → N-alpha-Acetyl-L-2.4-diaminobu-tyrate	Alternative acetylation	No

37.	R28	2-Oxoglutarate → R-2-Hydroxyglutarate	Reductive metabolism	No
38.	R29	R-2-Hydroxyglutarate → 2-Oxoglutarate	Oxidative metabolism	No
39.	R30	2-Oxoglutarate → 2-Oxohept-2-enedioate	Aromatic degradation link	No
40.	R31	2-Oxohept-2-enedioate → 2-Oxoglutarate + Acetyl-CoA	Aromatic degradation link	No
41.	R32	2-Oxohept-2-enedioate → 2-Hydroxyhepta-2,4-dienedioate	Aromatic degradation link	No
42.	R33	2-Hydroxyhepta-2,4-dienedioate → 2-Carboxy-2-oxohept-3-enedioate	Aromatic degradation link	No
43.	R34	2-Carboxy-2-oxohept-3-enedioate → 2-Carboxymethyl-2-hydroxymuconate	Aromatic degradation link	No
44.	R35	2-Carboxymethyl-2-hydroxymuconate → 2-Hydroxy-5-carboxymethylmuconate semialdehyde	Aromatic degradation link	No
45.	R36	2-Hydroxy-5-carboxymethylmuconate semialdehyde → 2-Oxoglutarate + Pyruvate	Aromatic degradation link	No
46.	R37	3,4-Dihydroxyphenylacetate → 2-Hydroxy-5-carboxymethylmuconate semialdehyde	Aromatic ring cleavage	No
47.	R38	S-3-Hydroxybutanoyl-CoA → Crotonyl-CoA	Fatty acid beta-oxidation	No
48.	R39	Crotonyl-CoA → 3-Hydroxybutanoyl-CoA	Fatty acid synthesis/degradation	No
49.	R40	Glutaryl-CoA → Glutaconyl-CoA to Crotonyl-CoA	Lysine degradation to TCA	Yes

R = reaction.

Supplementary material Table S3

Table S3. Stoichiometric structure.

Metabolite	N Reactions	N Consumed	N Produced	Net Balance	Role
L-aspartate	6	4	2	-2	Central metabolite
L-pipecolate	2	1	1	0	Balanced
L-aspartate 4-semialdehyde	2	2	0	-2	Primarily consumed
Acetyl-CoA	2	0	2	2	Primarily consumed
L-Isoleucine	1	1	0	-1	Primarily consumed
6-amino-2-oxohexanoate	1	1	0	-1	Primarily consumed
L-Leucine	1	1	0	-1	Primarily consumed
N-alpha-acetyl-L-2.4-diaminobutyrate	1	1	0	-1	Primarily consumed
L-Lysine	1	0	1	1	Primarily produced
L-Glutamate	1	0	1	1	Primarily produced
L-Homoserine	1	0	1	1	Primarily produced
Guanine	1	0	1	1	Primarily produced
Uracil	1	0	1	1	Primarily produced
Urea	1	0	1	1	Primarily produced

Supplementary material Table S4

Table S4. Reaction flux and stress.

R	Pathway	Flux B	Flux P	Flux change B	Flux change P	Stress score B	Stress score P	Stress category B	Stress category P
R1a	TCA cycle	5.8	48.09	-94.2	-51.91	94.2	51.91	Severe	High
R1b	TCA cycle	5.8	48.09	-94.2	-51.91	94.2	51.91	Severe	High
R1c	TCA cycle	5.8	48.09	-94.2	-51.91	94.2	51.91	Severe	High
R1d	TCA cycle	5.8	48.09	-94.2	-51.91	94.2	51.91	Severe	High
R2	Reductive	59.35	58.24	-40.65	-41.76	40.65	41.76	Moderate	Moderate
R3	Oxidative	38.02	111.73	-61.98	11.73	61.98	11.73	High	Low
R4	CoA-dependent	30.22	31.91	-69.78	-68.09	69.78	68.09	High	High
R5	Transamination	31.91	52.77	-68.09	-47.23	68.09	47.23	High	Moderate
R6a	Biosynthesis	67.9	90.32	-32.08	-9.68	32.08	9.68	Moderate	Low
R6b	Biosynthesis	67.9	90.32	-32.08	-9.68	32.08	9.68	Moderate	Low
R7	Degradation	114.16	37.47	14.16	-62.53	14.16	62.53	Low	High
R8	Oxidation/Transamination	30.22	31.91	-69.78	-68.09	69.78	68.09	High	High
R9	Decarboxylation	114.16	37.47	14.16	-62.53	14.16	62.53	Low	High
R10	Reductive amination	114.16	37.47	14.16	-62.53	14.16	62.53	Low	High
R11	Oxidative deamination	114.16	37.47	14.16	-62.53	14.16	62.53	Low	High