

Supplemental Material

Table S1. Soil chemistry of the litter layer versus the O horizon.

	Litter Layer	O Horizon
pH	4.3	4.9
%OM	27.4	25.8
Total C	0.7932	0.6292
Total N	26.02	19.60
C:N ratio	32.80	31.15
NO₃-N	2.2	3.7
P	61.3	57.3
Type	loam	loam

Table S2. Average number of reads assigned to nitrogen cycle pathway genes for each set of samples (n=4). The number of N cycle pathway reads were lower in the metatranscriptome (MT) than metagenome (MG) samples, and lower in the litter layer (LL) than the O-horizon (OH) samples.

Nitrogen Pathway	Gene	OH Initial	MT	OH +H2O	MT	OH +H2O +N	MT	LL Initial	MT	LL Control	MT	LL +H2O	MT	LL +H2O +N	MT	LL +H2O	MG	LL +H2O +N	MG
Nitrification	hao	1.25		1.5		0.5		0		0		0		0.25		1.5		2.25	
Denitrification	nosZ	3.25		0.75		2.25		1		0		0		0		3.25		5	
Denitrification	norB	9.5		3.25		6.75		0.5		0.5		0		0		51.5		61.25	
Denitrification	norC	0		0		0		0		0		0		0		1		2	
Denitrification	nirK	0		0		0		0		0		0		0		0		0	
Dissimilatory nitrate reduction	nirB	26.5		9.75		18.75		9.5		1		2.25		0.5		1452.75		1191.75	
Dissimilatory nitrate reduction	nirD	8.5		6.5		7		1		1		0.75		1.25		92.5		84	
Assimilatory nitrate reduction	nirA	142.5		51		110.25		29.5		6.5		7.5		7		1554.75		1436.75	
Assimilatory nitrate reduction	napB	0.75		0.5		0		1.75		0		0		0		12.5		11.75	
Nitrogen Fixation	nifH	3		0		0.5		0.75		1.5		0		0		1.75		1	

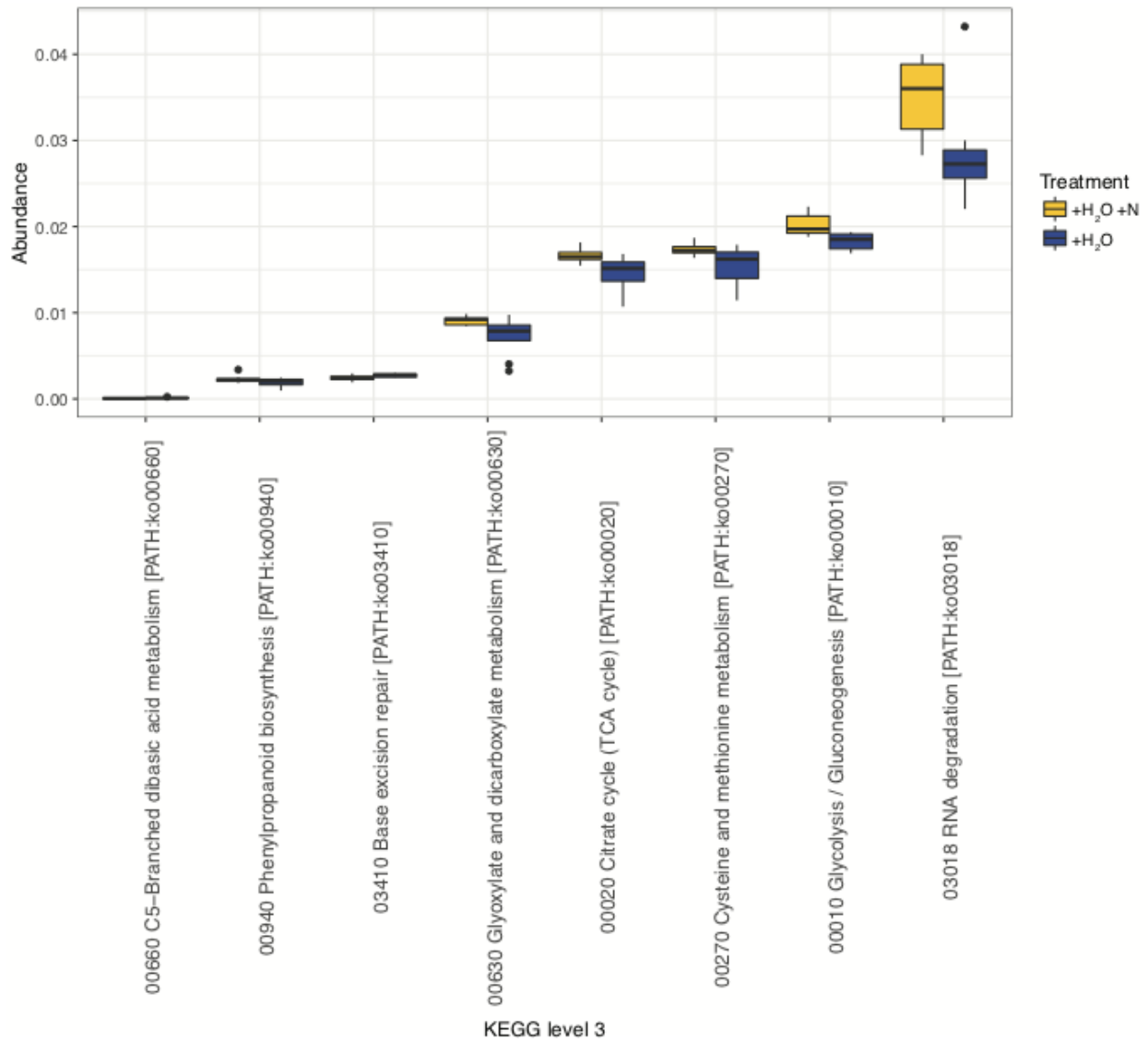


Figure S1. Carbohydrate metabolism pathways (KEGG annotations) with significantly different expression in the + H₂O treatment (blue) compared to the +H₂O +N treatment (orange) at the final sampling time (64 hours).

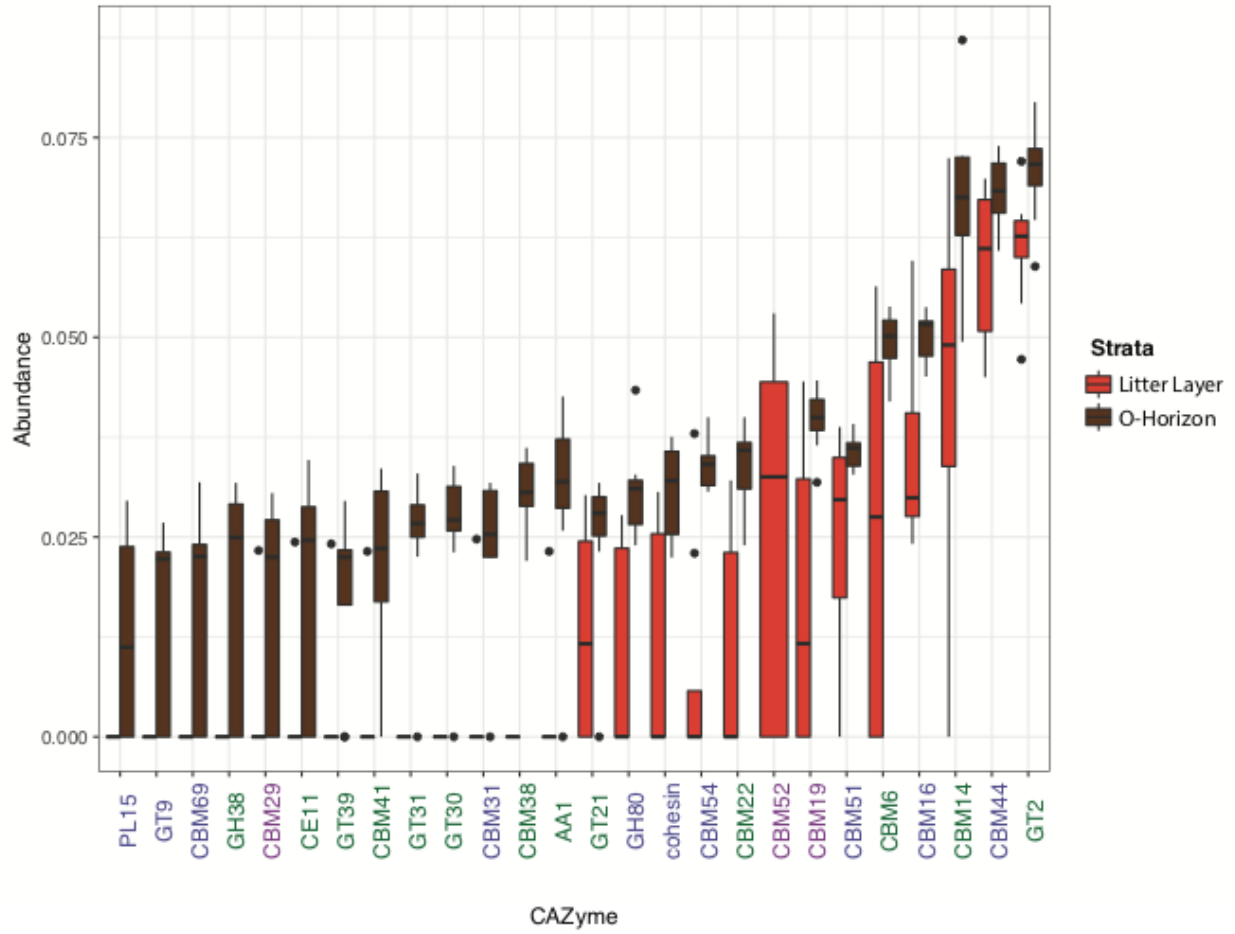


Figure S2. CAZymes with significantly different expression in the litter layer (red) compared to the O-horizon (brown) at the final sampling time (64 hours). CAZymes associated with bacteria/archaea (blue), fungi (purple) or both bacteria/archaea and fungi (green).

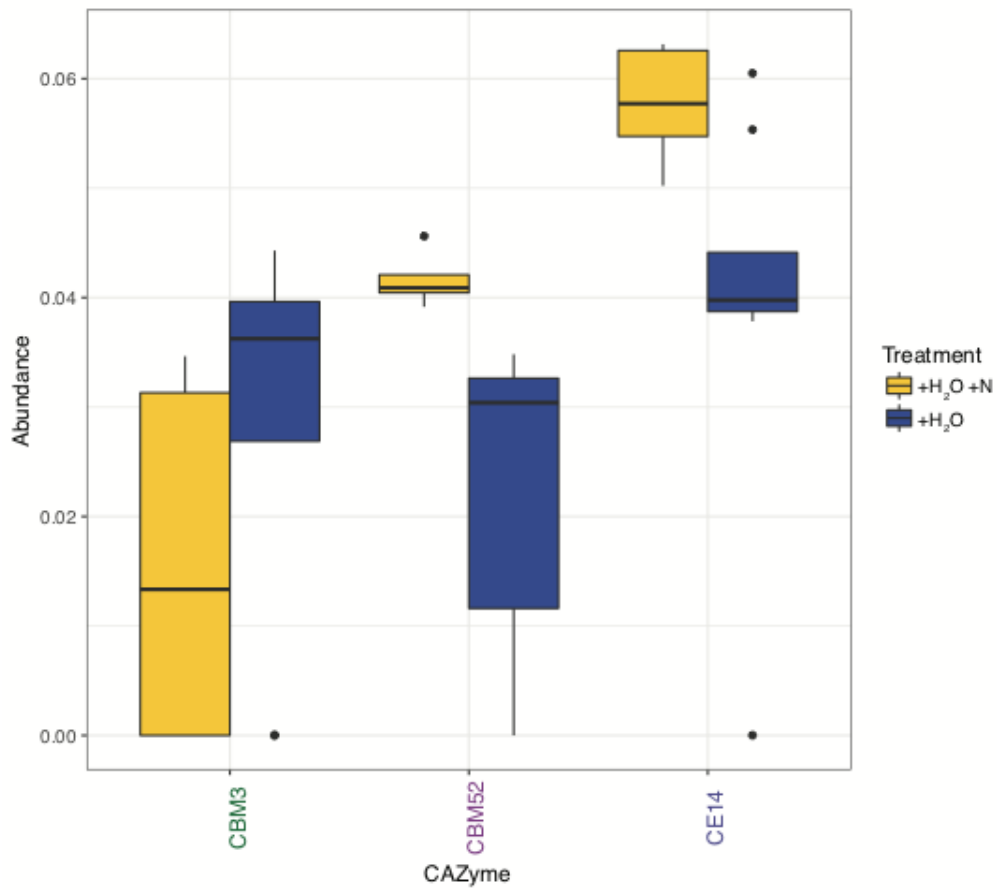


Figure S3. CAZymes with significantly different expression in the + H₂O treatment (blue) compared to the +H₂O +N treatment (orange) at the final sampling time (64 hours). CAZymes associated with bacteria/archaea (blue), fungi (purple) or both bacteria/archaea and fungi (green).

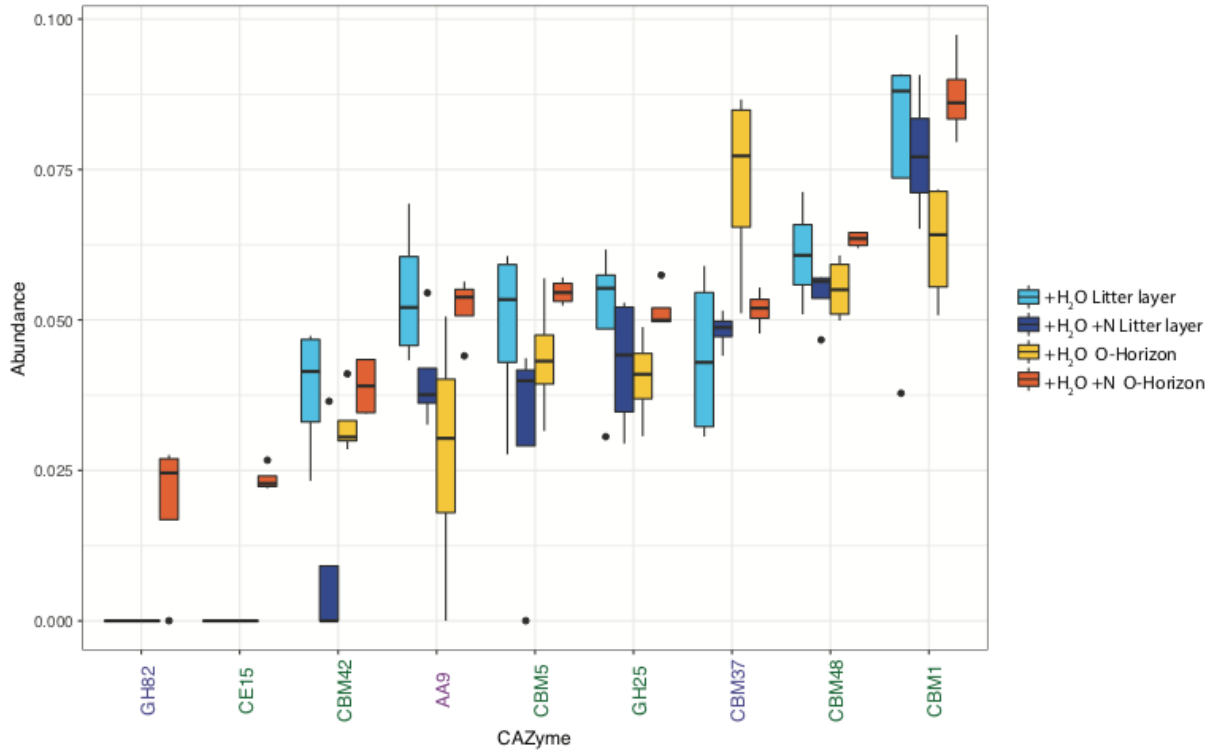


Figure S4. CAZymes showing a significant interactive effect between horizon and treatment [+ H₂O litter layer (light blue), +H₂O +N litter layer (dark blue), + H₂O O-horizon (light orange), +H₂O +N litter layer (dark orange)] at the final sampling time (64 hours). CAZymes associated with bacteria/archaea (blue), fungi (purple) or both bacteria/archaea and fungi (green).

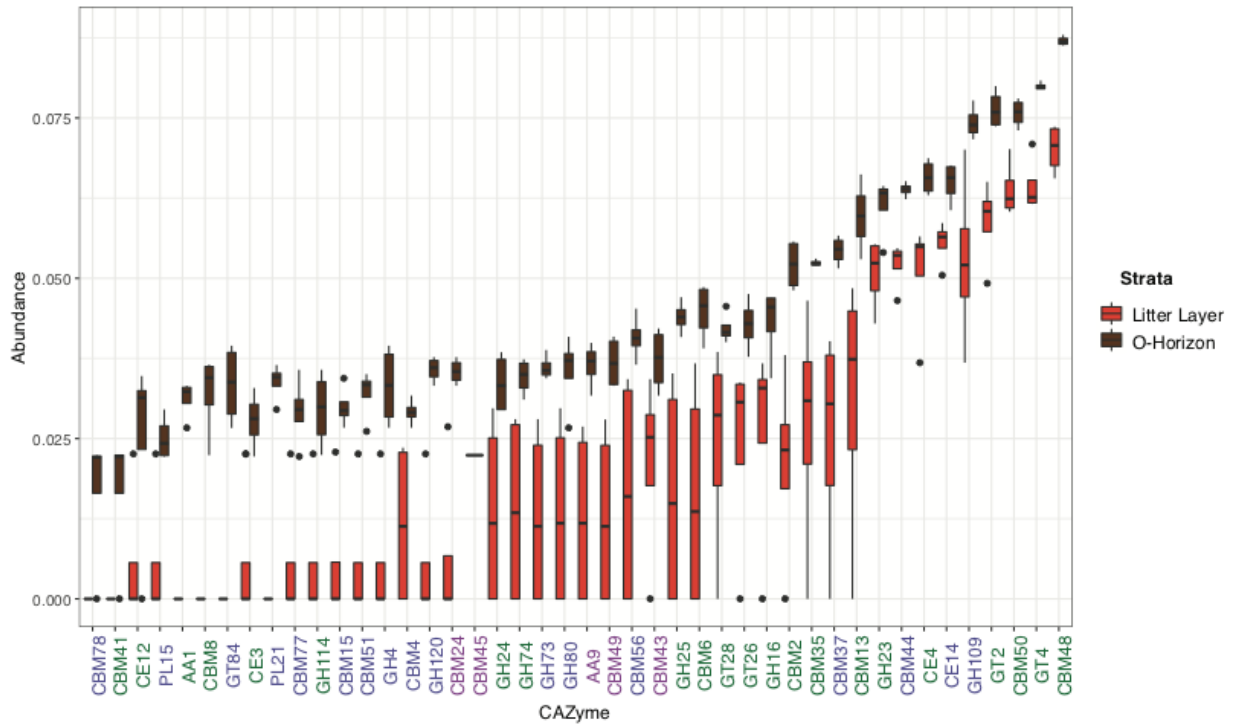
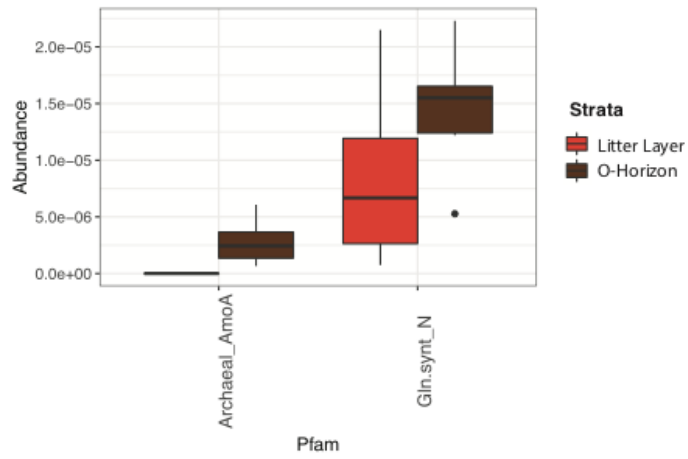


Figure S5. CAZymes with significantly different expression at the initial sampling time (0 hours) in the litter layer (red) compared to the O-horizon (brown). CAZymes associated with bacteria/archaea (blue), fungi (purple) or both bacteria/archaea and fungi (green).

A.



B.

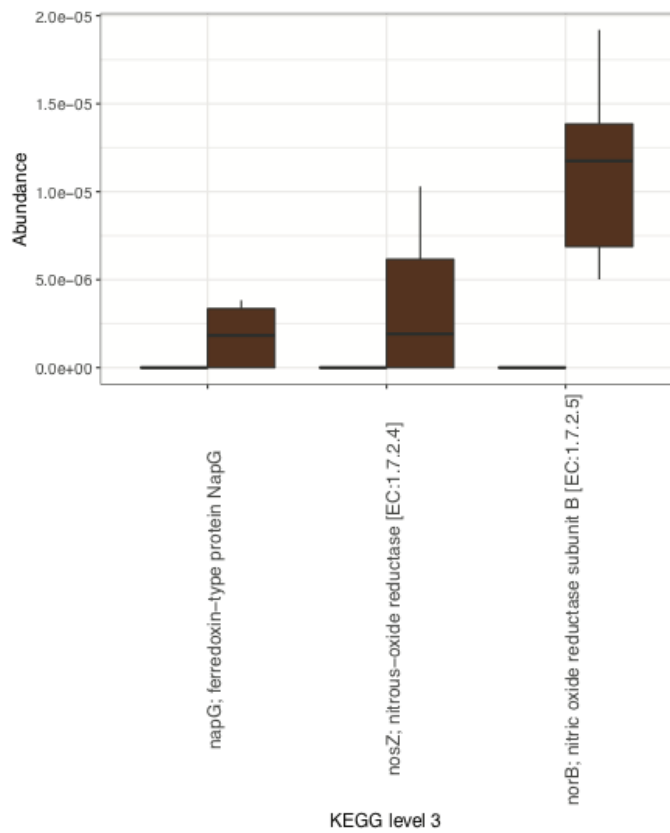


Figure S6. Nitrogen cycling genes showing significantly different expression levels in the litter layer compared to the O-horizon as annotated by a) pFam b) KEGG.