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Organic and inorganic nitrogen amendments reduce biodegradation of biodegradable plastic mulch films

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Table S1: Enzymes assayed before and after 16-week incubation.

Abbreviation	Enzyme name	Target substrate in natural environment	Substrate used in experiment	Indicator of microbial activity
BG	β-glucosidase	sugar	4-MUB-β-D-glucopyranoside	Carbon cycling
NAG	N-acetyl β-glucosaminidase	chitin	4-MUB-N-acetyl-β-D-glucosaminide	Carbon and nitrogen cycling
CB	β-D cellubiosidase	cellulose	4-MUB-β-D-cellobioside	Carbon cycling

MUB = 4-methylumbelliferone; MUC = 7-amino-4-methylcoumarin

Table S2: F values from three-way ANOVAs showing effects of location, nitrogen and plastic treatment on soil chemical and biological characteristics. Significant differences are in bold; *p < 0.05; **p < 0.01; ***p < 0.001.

Factor	CO_2-C (µg C g^{-1} dry soil)	NH_4 (µg NH_4 g^{-1} dry soil)	NO_3 (µg NO_3 g^{-1} dry soil)	%C	%N	C:N	Log (amoA gene copies g^{-1} dry soil)	Log (fungal gene copies g^{-1} dry soil)	Log (bacterial gene copies g^{-1} dry soil)
Location	**39.95*** **	**643.26*** **	**5.01***	**1223.33*** **	**834.61*** **	**141.59*** **	**489.92*** **	**120.31*** **	**18.08*** **
Nitrogen	**39.49*** **	**133130*** **	**119.57*** **	**7.78*** **	**16.35*** **	**6.91****	**3.31***	**14.82*** **	**12.43*** **
Plastic	**461.34*** **	9.52E-01	2.57	0.04	1.36	1.88	**10.06****	3.22	**13.63*** **
Location:Nitrogen	**6.09****	**542.63*** **	**3.61***	**4.48****	2.47	2.48	**6.45****	1.42	**14.25*** **
Location:Plastic	**31.71*** **	3.05E-01	0.39	4.11	**5.95***	0.03	2.49	1.50	0.85
Nitrogen:Plastic	**11.81*** **	1.46E+00	0.07	**3.47***	2.64	0.80	**3.30***	**5.60****	**12.20*** **
Location:Nitrogen:Plastic	**4.68****	8.13E-01	0.21	0.77	0.31	0.62	**3.87***	2.07	**15.67*** **

Table S3: C:N ratios, %C and %N of soil before (T0) and after 16-week incubation. TN: Tennessee, WA: Washington. T0: initial soils, prior to experimental amendments. Mean (n=3) ± standard error of the mean (SE).

Location	Mulch	Nitrogen	C:N ratio	%C	%N
TN T0			**9.43 ± 0.01**	**0.75 ± 0.01**	**0.08 ± 0.001**
TN	no	Amino acid	8.33 ± 0.08	0.69 ± 0.02	0.08 ± 0.001
TN	no	Ammonium nitrate	8.02 ± 0.06	0.65 ± 0.02	0.08 ± 0.002
TN	no	No nitrogen	8.84 ± 0.14	0.70 ± 0.03	0.08 ± 0.003
TN	no	Urea	8.25 ± 0.26	0.72 ± 0.02	0.09 ± 0.001
TN	yes	Amino acid	8.46 ± 0.13	0.72 ± 0.01	0.09 ± 0.002
TN	yes	Ammonium nitrate	8.12 ± 0.12	0.68 ± 0.02	0.08 ± 0.002
TN	yes	No nitrogen	8.93 ± 0.16	0.73 ± 0.03	0.08 ± 0.002
TN	yes	Urea	8.25 ± 0.15	0.70 ± 0.00	0.09 ± 0.001
WA T0			**10.69 ± 0.31**	**1.23 ± 0.06**	**0.12 ± 0.003**
WA	no	Amino acid	9.23 ± 0.05	1.11 ± 0.01	0.12 ± 0.001
WA	no	Ammonium nitrate	9.52 ± 0.08	1.14 ± 0.01	0.12 ± 0.001
WA	no	No nitrogen	9.70 ± 0.13	1.09 ± 0.01	0.11 ± 0.002
WA	no	Urea	9.37 ± 0.11	1.22 ± 0.03	0.13 ± 0.004
WA	yes	Amino acid	9.40 ± 0.03	1.12 ± 0.02	0.12 ± 0.001
WA	yes	Ammonium nitrate	9.39 ± 0.02	1.12 ± 0.01	0.12 ± 0.001
WA	yes	No nitrogen	10.06 ± 0.17	1.11 ± 0.02	0.11 ± 0.001
WA	yes	Urea	9.37 ± 0.17	1.13 ± 0.02	0.12 ± 0.001

Table S4: Percent biodegradation of BioAgri mulch after 16 weeks. TN: Tennessee, WA: Washington.

Treatment	Location	Theoretical mulch CO_2-C released (µg mulch-C g^{-1} dry soil)	Theoretical biodegradation (%)
Urea	TN	49	4
Amino acid	TN	83	6
Ammonium nitrate	TN	74	6
No Nitrogen	TN	132	10
Urea	WA	47	4
Amino acid	WA	46	4
Ammonium nitrate	WA	41	3
No Nitrogen	WA	63	5

Total amount of carbon added in the form of plastic at t=0 was 1309 µg C g^{-1} dry soil for TN jars and 1317 µg C g^{-1} dry soil for WA jars.

Table S5. Thermogravimetric analysis (TGA) heating stage temperatures and % mass remaining of BioAgri mulch at 600ºC. TN: Tennessee, WA: Washington.

Treatment	**Temp (°C)**					**Mass Remaining**
	$T_{0,A}$, ºC	**$T_{max,A}$, ºC**	**$T_{0,B}$, ºC**	**$T_{max,B}$, ºC**	**$T_{f,B}$, ºC**	**(%)**
Control *	292.58	331.47	389.75	411.36	433.17	10.48
Amino acid, TN	297.05	331.21	390.42	412.57	431.69	19.94
Amino acid, WA	293.19	327.42	384.03	406.11	429.19	36.81
Ammonium Nitrate, TN	291.96	331.01	387.22	408.12	429.99	32.64
Ammonium Nitrate, WA	296.23	328.75	390.76	410.69	430.48	17.29
No Nitrogen, TN	296.81	333.28	388.39	410.88	430.88	32.02
No Nitrogen, WA	290.55	327.39	391.33	410.92	432.10	22.05
Urea, TN	295.50	329.18	385.76	404.45	429.97	27.96
Urea, WA	295.42	327.86	392.36	410.88	431.87	17.77

Subscripts for *Ti,j*: *0* = onset temperature *max* = maximum (most rapid weight loss) temperature, *f*= final temperature for heating stage *i* (A=starch and B=PBAT)**.** % Mass remaining = % inorganics present (e.g., binders: $CaCO_3$ or nanoclays) or gels. One would expect that as biodegradation (of the polymers) occurs, the measured mass loss will decrease; i.e., the % of inorganics would increase. *Agriculturally-weathered BioAgri: Taken from Hayes et al. (2017).

Table S6. Change in molecular weight-related properties of BioAgri mulch during incubation as per gel permeation chromatographic (GPC) analysis. TN: Tennessee, WA: Washington.

N Amendment	Location			
	TN		WA	
	M_w, kDa	PDI	M_w, kDa	PDI
Control*	189 + 12	2.51 + 0.06	189 + 12	2.51 + 0.06
No nitrogen	161 + 2	4.34 + 0.36	151 + 5	2.83 + 0.39
Urea	151 + 3	2.76 + 0.07	215 + 1	2.09 + 0.12
Amino acid	151 + 2	2.64 + 0.19	157 + 2	2.29 + 0.32
Ammonium nitrate	152 + 2	2.51 + 0.13	169 + 2	2.13 + 0.04

M_w = weight-averaged molecular weight (in kDa), PDI = polydispersity index; error bars represent standard deviation; values are based on 2 replicates. *Agriculturally-weathered BioAgri: Taken from Hayes et al. (2017).

Table S7. Peak assignments for FTIR analysis for BioAgri mulch (Hayes et al., 2017).

Wavenumber (cm^{-1})	Contribution
2956, 2920, 2876, 2846	C-H stretch
1712	C=O stretch (polyester)
1646	C=O stretch (polysaccharide)
1456, 1410, 1390	$-CH_2-$ bend
1268, 1252, 1166, 1118, 1102, 1082	C-O stretch (polyester)
1076-1000	C=C for PBAT (via environmental weathering)
874	C-H stretch
728	$(CH_2)_4$ bend

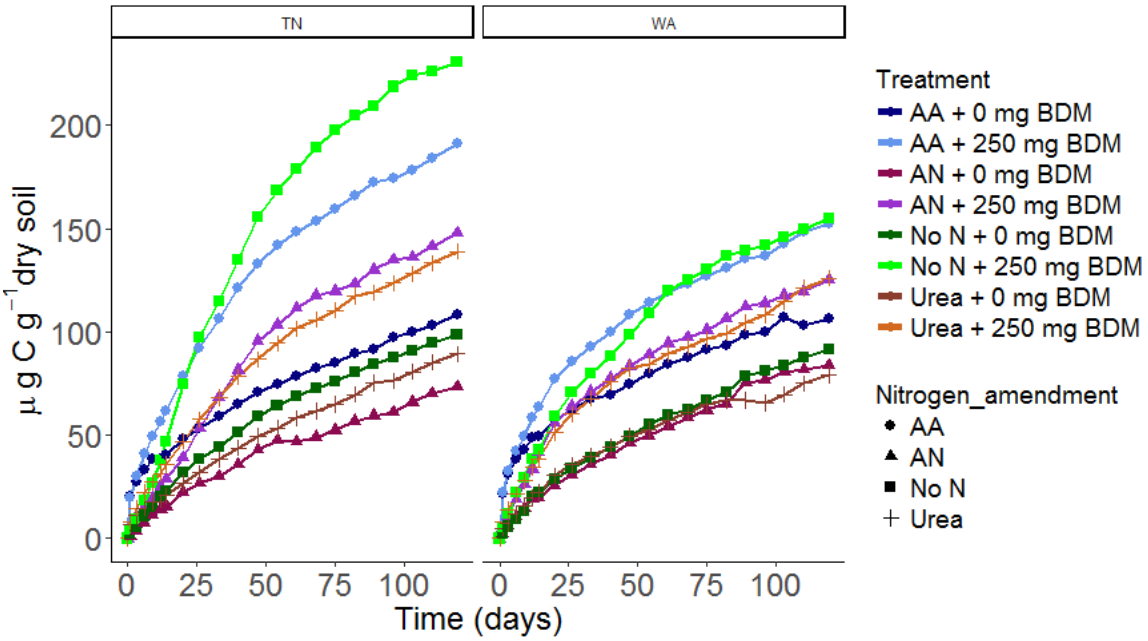

Figure S1: Cumulative CO_2-C released over 16 weeks. Each data point represents a mean of 3 replicate microcosms. TN: Tennessee, WA: Washington. AA: amino acid, AN: ammonium nitrate.

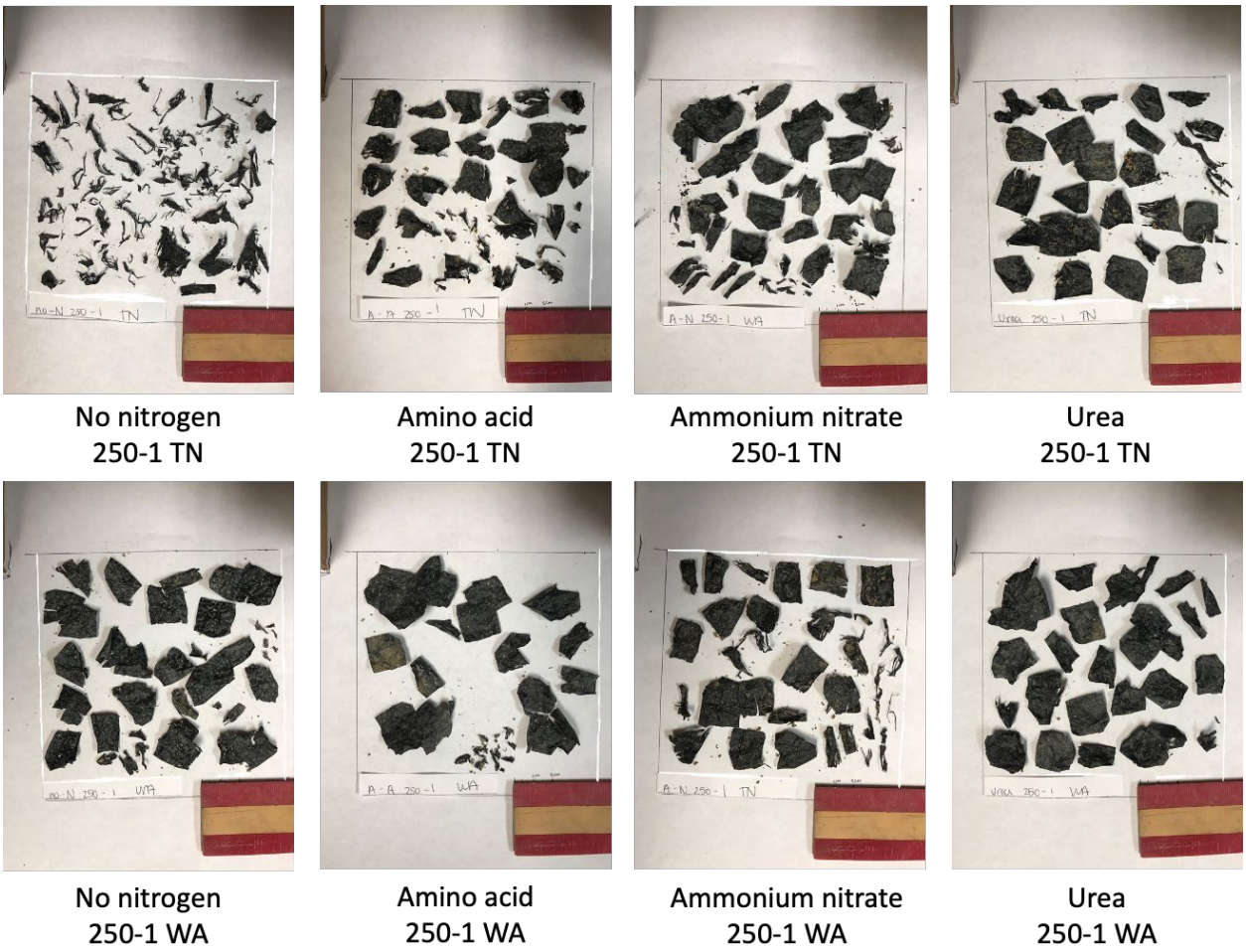

Figure S2: Visualization of plastic pieces after 16 weeks incubation (raw images). All images were taken from mulches from the first replicate microcosm for each treatment. TN: Tennessee, WA: Washington.

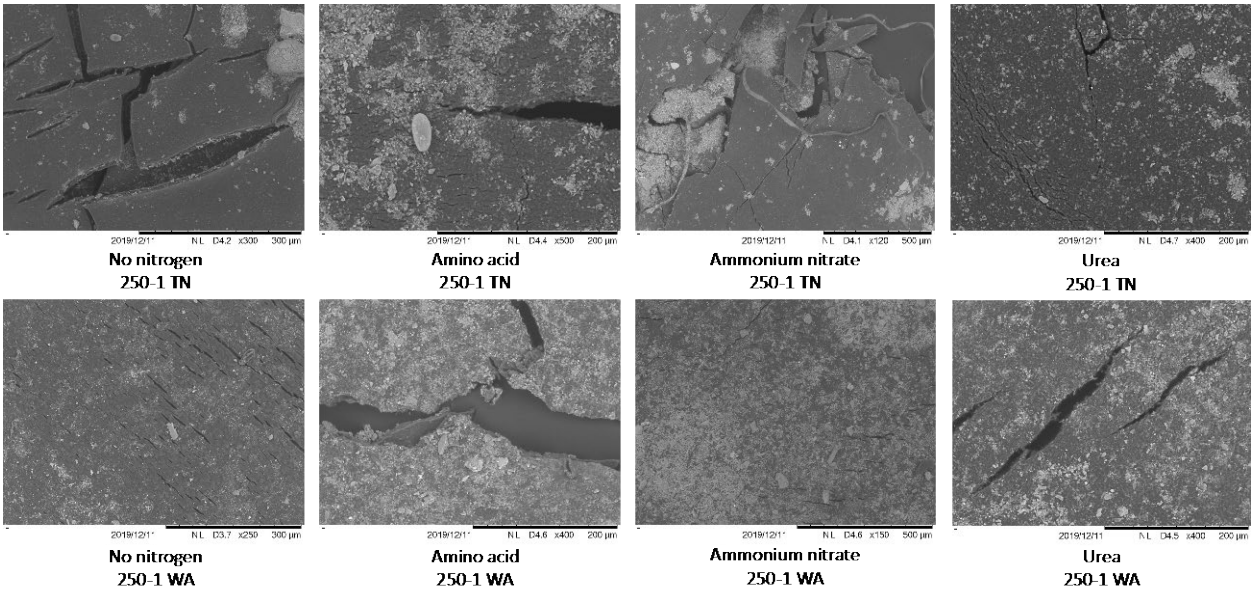

Figure S3: Scanning electron microscopy (SEM) images of plastic mulches after incubation of 16 weeks. All images were taken from mulches from the first replicate microcosm for each treatment. TN: Tennessee, WA: Washington.

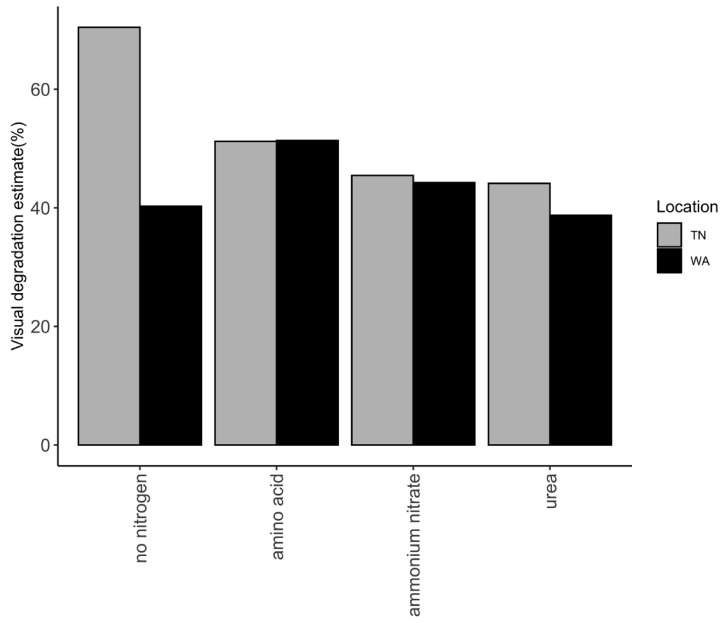

Figure S4: Percent biodegradation values as estimated by calculating the remaining surface area of mulch pieces after 16-week incubation. Only one rep was visualized per nitrogen treatment. Calculations for surface area done using ImageJ. TN: Tennessee, WA: Washington.

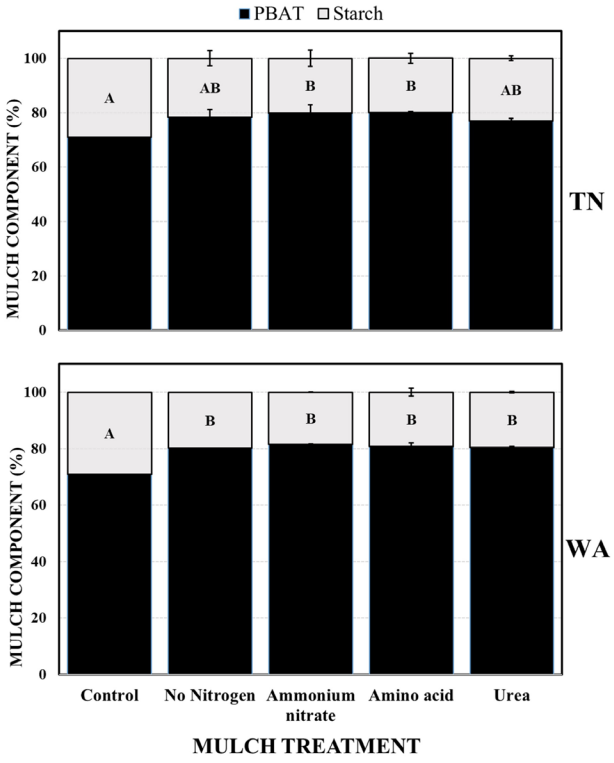

Figure S5. Starch and PBAT components for BioAgri after 16 weeks of incubation. Mulch component (%) was determined 20°C lower before the onset of degradation process (T_0, Table S6) and 20°C higher after the rapid degradation process (T_{max}, Table S6) of each mulch treatment. TN: Tennessee, WA: Washington. Bar plot represents mulch component (%) and error bars reflect standard deviation (where n=2). *Control* refers to agriculturally-weathered BioAgri plastic mulch samples in TN, taken from Hayes et al. (2017).

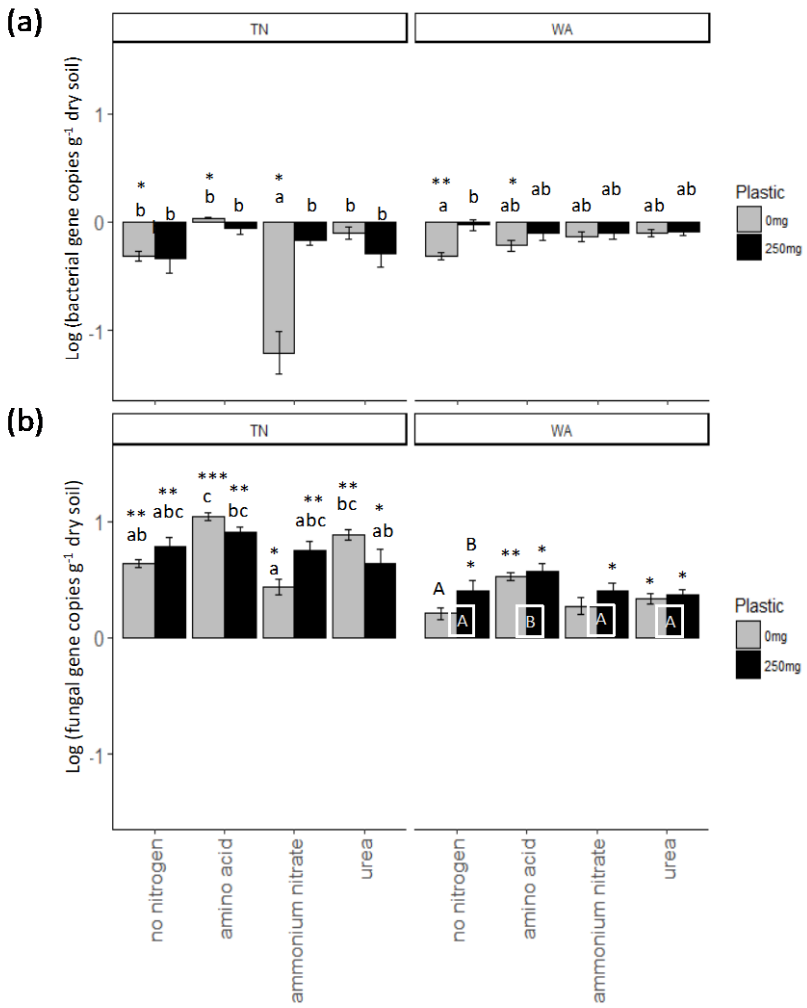

Figure S6: Changes in (a) bacterial and (b) fungal gene abundances over 16 weeks. All gene abundances were log transformed, then abundances in initial soils subtracted from final (16 week) samples. Each bar represents a mean of 3 replicate microcosms and error bars are standard error. Lowercase letters indicate interaction effects at $\alpha \leq 0.05$ for bacterial abundance in TN and WA and fungal abundance in TN. Uppercase letters for fungal abundance in WA along x-axis indicate a significant main effect of nitrogen treatment at $\alpha \leq 0.05$. Uppercase letters above bars for fungal gene abundance in WA indicate a significant main effect of plastic at $\alpha \leq 0.05$. Asterisks indicate significant increase or decrease in gene abundance from t = 0 as per a *t*-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. TN: Tennessee, WA: Washington.

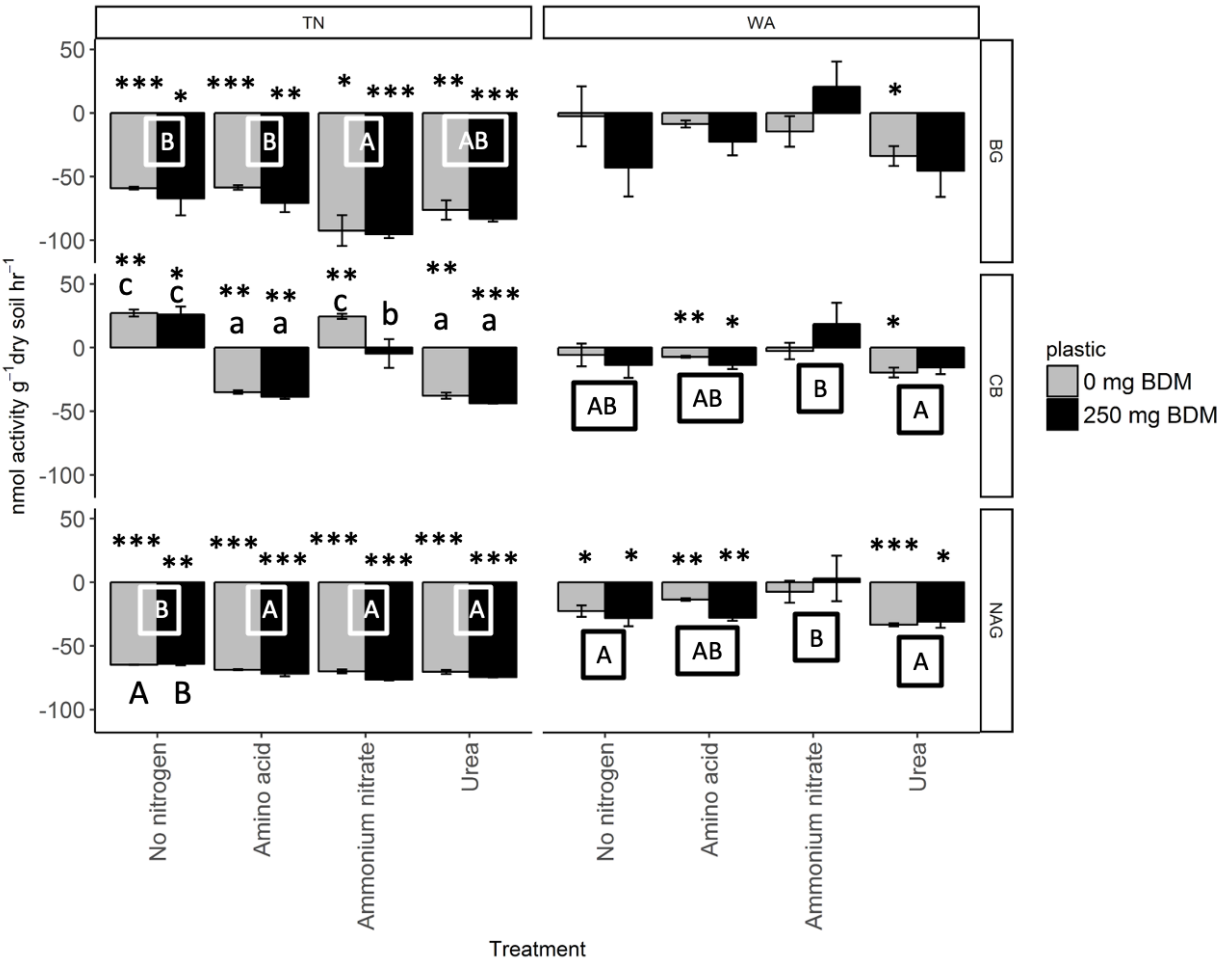

Figure S7: Changes in enzyme activities of β- glucosidase (BG), cellubiosidase (CB), and N-acetyl β-D- glucosaminidase (NAG) after 16 weeks. Each bar represents a mean of 3 replicate microcosms and error bars are standard error. Lowercase letters indicate a significant interaction effect at $\alpha \leq 0.05$. Boxed uppercase letters indicate a significant main effect of nitrogen at $\alpha \leq 0.05$. Unboxed uppercase letters indicate a significant main effect of plastic at $\alpha \leq 0.05$. Asterisks indicate significant increase or decrease in enzyme activity from t = 0 based on a *t*-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. TN: Tennessee, WA: Washington.

References

Hayes, D. G., Wadsworth, L. C., Sintim, H. Y., Flury, M., English, M., Schaeffer, S., and Saxton, A. M.: Effect of diverse weathering conditions on the physicochemical properties of biodegradable plastic mulches, Polymer Testing, 62, 454-467, https://doi.org/10.1016/j.polymertesting.2017.07.027, 2017.