

SUPPLEMENTARY INFORMATION

Title: Biochar and denitrification in soils: when, how much and why does biochar reduce N₂O emissions?

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Table S1. N₂O concentrations in doubly autoclaved soils (with and without additions of 2% of Brush biochar) before and after a spike with KNO₃ (50 mg N kg⁻¹ soil).

Treatment	Average N₂O concentration in the jars' headspace (ppmv)*	Average N₂O concentration in the jars' headspace (ppmv) after addition of KNO₃**
Elba (control)	0.346 (0.059)	0.412 (0.002)
Elba (2% biochar)	0.363 (0.018)	0.406 (0.003)
Guarapuava (control)	0.375 (0.009)	0.395 (0.002)
Guarapuava (2% biochar)	0.376 (0.008)	0.397 (0.006)
Average N₂O concentration in lab air	0.387 (0.021)	0.404 (0.011)

Standard deviation in brackets.

*after a closing period of 40 min. Measured 12 h after autoclaving.

** after a closing period of 40 min. Measured 12 h after autoclaving and immediately after KNO₃ addition.

Table S2. Main chemical properties of the biochars selected for the experiments

Biochar	Feedstock	C (%)	N (mg kg ⁻¹)	C/N	pH	EC (dS m ⁻¹)	NH ₄ ⁺ NO ₃ ⁻		S-BET (m ² ·g ⁻¹)	Volatile matter	Ash (%)	Fixed C
							(mg kg ⁻¹)					
Grass clippings	Summer yard waste (mainly grass)	53.5	49.5	10.8	9.45	6.66	12	3	50 ⁽¹⁾	38.4	25.5	59.8
Dairy manure	Raw dairy manure	51.2	21.0	24.4	10.68	6.02	5	3	13 ⁽²⁾	33.9	12.4	53.7
Leaves	Fall yard waste (mainly leaves)	60.7	11.3	53.5	9.06	0.58	n.d.	n.d.	n.m.	40.3	14.5	45.2
Bamboo	Chinese bamboo	60.5	5.5	110.0	7.33	n.m.	2	1	126 ⁽³⁾	n.m.	7.2 ⁽³⁾	n.m.
Hazelnut	Hazelnut shells	80.1	4.6	174.8	9.60	1.47	n.d.	1	6.8	37.2	1.9	60.9
Mixed woodchips	Chipped pallets	85.9	3.7	231.1	6.43	0.88	n.d.	1	n.m.	26.9	10.9	62.1
Oak	Oak wood	83.9	1.9	449.6	8.16	3.83	--	1	176	30.7	3.7	65.6
Brush	Winter yard waste (mainly brush)	84.0	1.4	609.5	6.78	0.12	n.d.	n.d.	10.0	40.1	1.8	58.2
Pine	Pine wood	83.4	1.0	859.4	6.86	0.08	n.d.	1	7.8	37.0	1.0	62.0

EC: electrical conductivity. S-BET: surface area (Brunauer–Emmett–Teller method⁽⁴⁾). Results expressed in dry weight basis. n.d.: not detected; n.m.: not measured

1. Keiluweit M, Nico PS, Johnson MG, & Kleber M (2010) Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ Sci Technol* 44(4):1247-1253.
2. Cao X & Harris W (2010) Properties of dairy-manure-derived biochar pertinent to its potential use in remediation. *Bioresour Technol* 101(14):5222-5228.
3. Mui ELK, Cheung WH, Valix M, & McKay G (2010) Dye adsorption onto char from bamboo. *J Hazard Mater* 177(1–3):1001-1005.
4. Brunauer S, Emmett PH, & Teller E (1938) Adsorption of gases in multimolecular layers. *J Am Chem Soc* 60(2):309-319.

Table S3. Main physical and chemical properties of selected soils.

Soil	Location: (Latitude Longitude)	pH	USDA texture classification	Sand (%)	Clay (%)	Bulk density* (kg dm ⁻³)	DOC DON NH ₄ ⁺ NO ₃ ⁻ (mg kg ⁻¹)			
							DOC	DON	NH ₄ ⁺	NO ₃ ⁻
Elba	43° 7' 48.2" -78° 6' 37.7"	5.60	Organic soil (muck)	n.m.	n.m.	0.65	966	21	59	81
Lins	-21° 38' 31.3" -49° 44' 25.6"	5.49	Sandy loam	82.1	13.4	1.55	54	n.d.	7	16
Arkport	42° 26' 30.3" -76° 28' 15.5"	7.14	Sandy loam	61.8	10.0	1.55	156	12	4	18
Lentiscosa	37° 48' 18.5" -1° 29' 35.6"	8.58	Sandy clay	47.3	15.1	1.33	64	n.d.	2	31
Tioga	42° 27' 11.6" -76° 27' 25.1"	5.95	Loam	39.7	14.6	1.45	68	3	3	10
Howard	42° 22' 35.4" -76° 23' 42.4"	6.80	Loam	42.6	15.4	1.45	76	3	3	23
Secanos	37° 45' 55.5" -1° 31' 33.3"	8.18	Loam	32.0	25.8	1.35	125	2	3	96
Cabezo	37° 47' 47.5" -1° 34' 53.4"	9.02	Loam	43.2	19.7	1.42	71	n.d.	6	24
Hudson A	42° 26' 34.8" -76° 28' 0.4"	5.84	Silt loam	23.6	18.9	1.38	174	8	8	4
Madalin	42° 26' 47.1" -76° 26' 26.0"	6.36	Silt loam	11.3	20.9	1.35	132	18	2	53
Niagara	42° 26' 52.6" -76° 27' 5.5"	6.47	Silt loam	17.9	16.3	1.39	85	1	3	78
Hudson B	42° 26' 32.2" -76° 27' 59.6"	7.19	Silt clay loam	5.2	27.8	1.29	103	14	6	9
Costa	37° 45' 55.5" -1° 31' 33.3"	8.10	Silt clay loam	13.3	32.4	1.28	219	135	11	1077
Coronela	37° 46' 36.6" -1° 32' 48.7"	7.86	Clay loam	29.3	27.8	1.34	142	n.d.	3	492
Guarapuava	-25° 17' 0.1" -51° 47' 58.0"	6.58	Silt clay	4.3	44.3	n.m.	150	7	22	30

*bulk densities were calculated from (1), with the exception of Guarapuava soil (due to its high clay content that does not allow the calculation) and the muck soil, which was measured in the field by soil coring.

DOC: dissolved organic C; DON: dissolved organic N, calculated by subtracting the sum of NH₄⁺ and NO₃⁻ from the total dissolved N.

n.m.: not measured; n.d.: not detected.

1. Saxton KE, Rawls WJ, Romberger JS, & Papendick RI (1986) Estimating generalized soil-water characteristics from texture. *Soil Sci Soc Amer J.* 50(4):1031-1036.

Table S4. pH in Elba soil observed at days 1, 3 and 14 and average over time after addition of the different biochars.

	pH (day 1)	std dev	pH (day 3)	std dev	pH (day 14)	std dev	Average pH (over time)
Control	5.67	0.02	5.55	0.04	5.59	0.05	5.60^a
Grass clippings	5.65	0.01	5.58	0.00	5.57	0.03	5.60^a
Dairy manure	5.78	0.02	5.72	0.01	5.71	0.04	5.73^b
Leaves	5.69	0.04	5.59	0.03	5.62	0.08	5.63^a
Bamboo	5.64	0.02	5.55	0.00	5.63	0.04	5.60^a
Hazelnut	5.65	0.02	5.56	0.02	5.65	0.04	5.62^a
Mixed woodchips	5.67	0.02	5.59	0.01	5.66	0.05	5.64^a
Oak	5.64	0.01	5.59	0.01	5.59	0.01	5.60^a
Brush	5.62	0.01	5.56	0.01	5.63	0.04	5.60^a
Pine	5.61	0.04	5.57	0.02	5.61	0.00	5.60^a

Means followed by standard deviations for each day (N=2). Repeated measures ANOVA was used to test the effect of biochar addition on soil pH over time (N=6). Since the assumption of sphericity was not validated, the Greenhouse-Geisser correction was applied. There was no time-treatment interaction (df (Greenhouse-Geisser) = 10.9; P= 0.244) and biochar additions had a significant effect (df=9; P=0.002) on pH. In the last column, values followed by a different letter are significantly different according to the Tukey post hoc test at P<0.05.

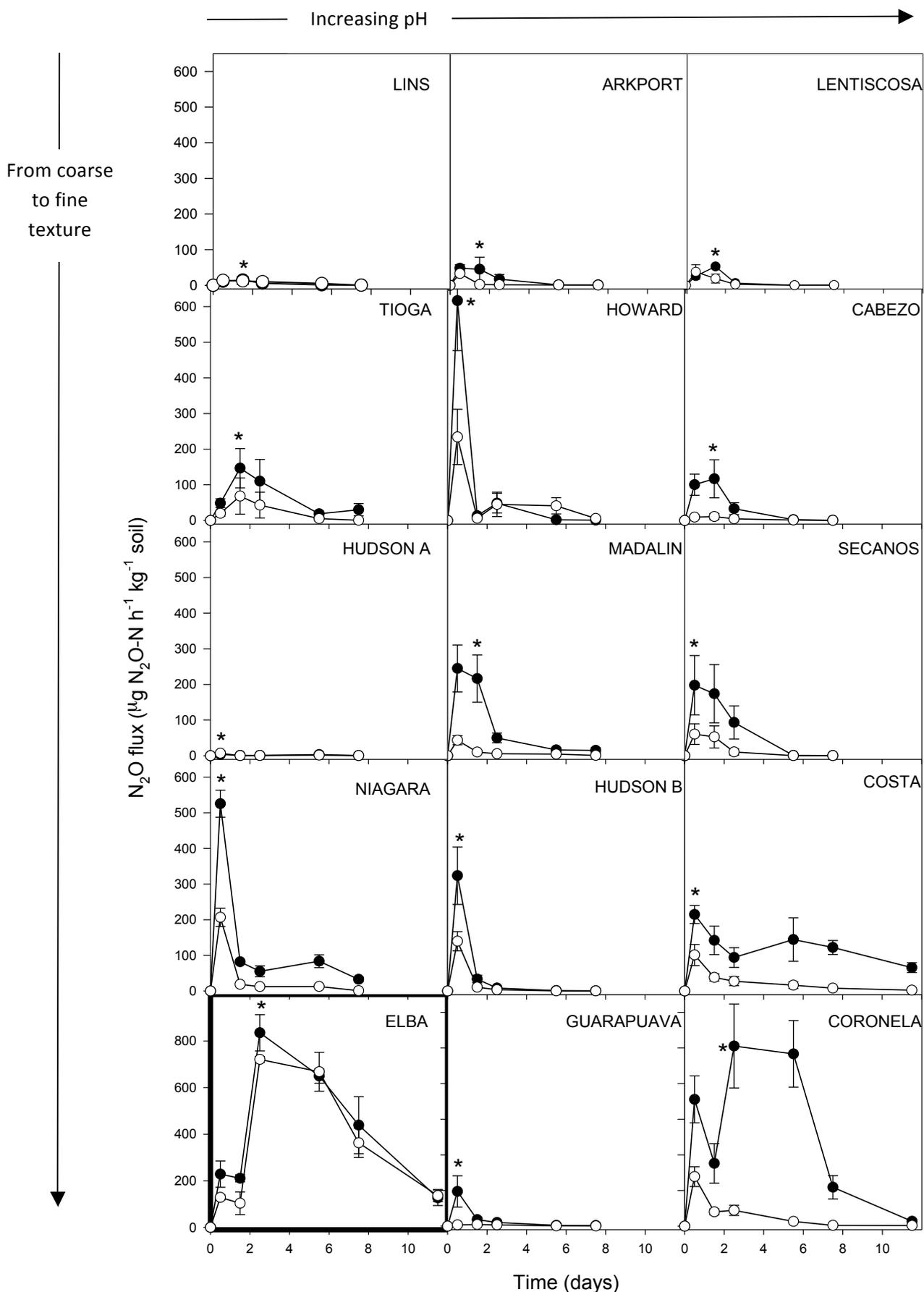


Fig. S1. N_2O fluxes from fifteen agricultural soils amended with 2% (dry weight basis) brush biochar (empty dots) in comparison to unamended soils (filled dots). Graphs are ordered with increasing soil pH from left to right and with finer texture from top to bottom. Note that the Elba soil is an exception (organic soil) therefore highlighted (thicker lines) and using a different y axis scale. Error bars represent the standard error of the mean (n=4). The asterisk in each graph points at the time when the differences between fluxes from soils amended with biochar in comparison to control soils were greatest (which usually corresponds with the peak of emissions). These samples were selected for analysis of N_2 and N_2O isotopic composition.

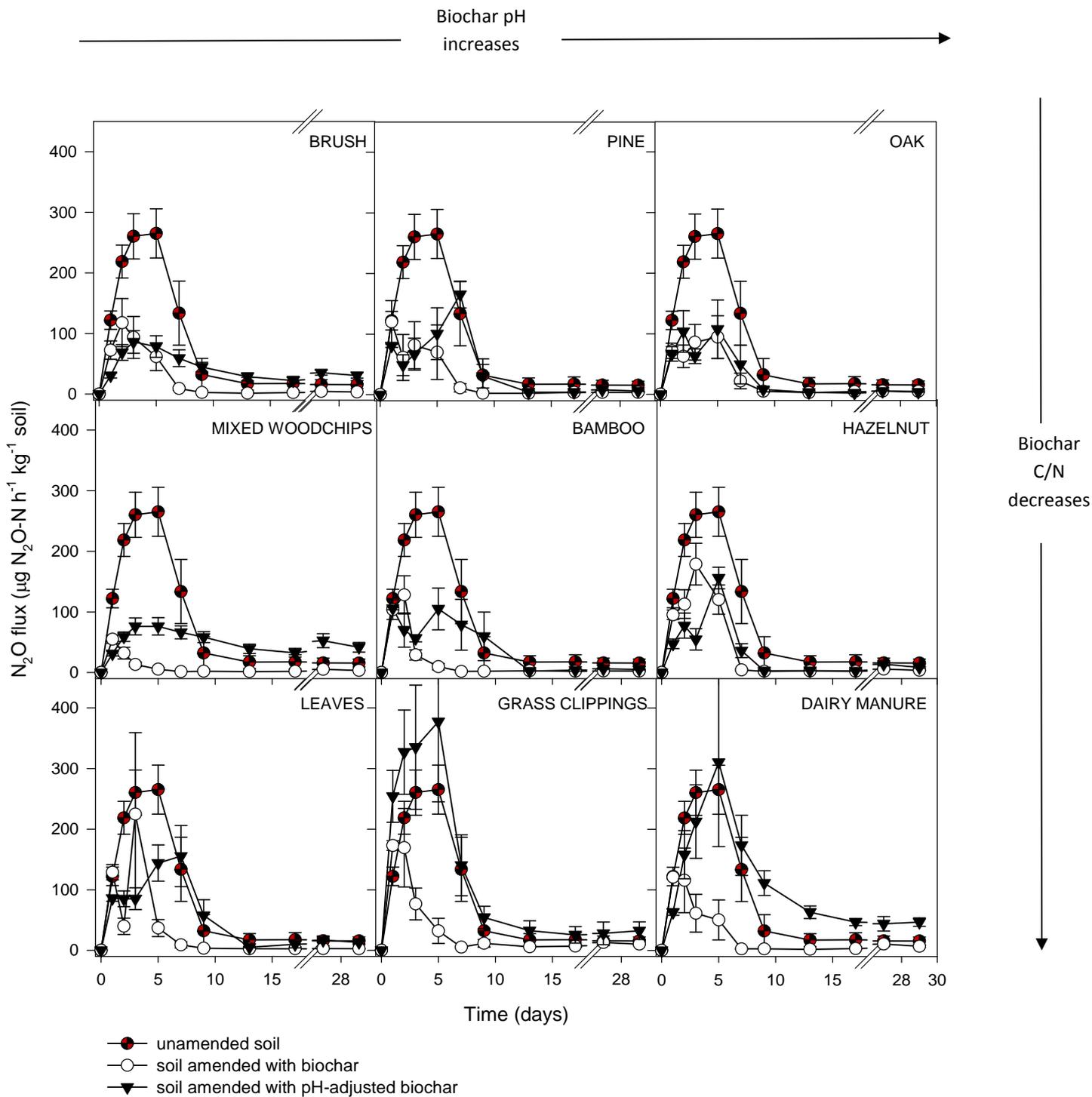


Fig. S2. N_2O fluxes from a muck soil amended with nine different biochars at 2% (dry weight basis) incubated under denitrification conditions (90% WFPS, 30 °C). Graphs are organized from left to right with increasing biochar pH and from top to bottom with decreasing C/N ratios. Error bars represent the standard error of the mean ($n=4$).

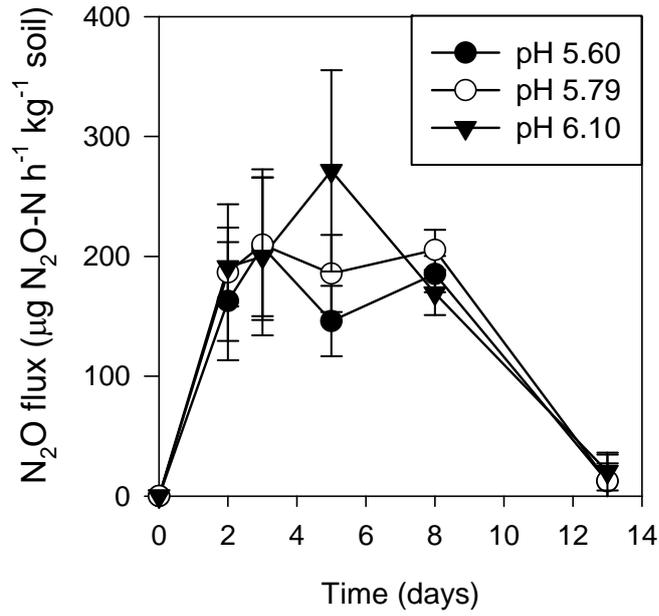


Fig. S3. N₂O fluxes from a muck soil (Elba) at its natural pH (5.60) and amended with CaCO₃ to increase its pH to 5.79 and 6.10. The soil was incubated under denitrification conditions (90% WFPS, 30°C). Error bars represent the standard error of the mean (n=4) for N₂O fluxes.

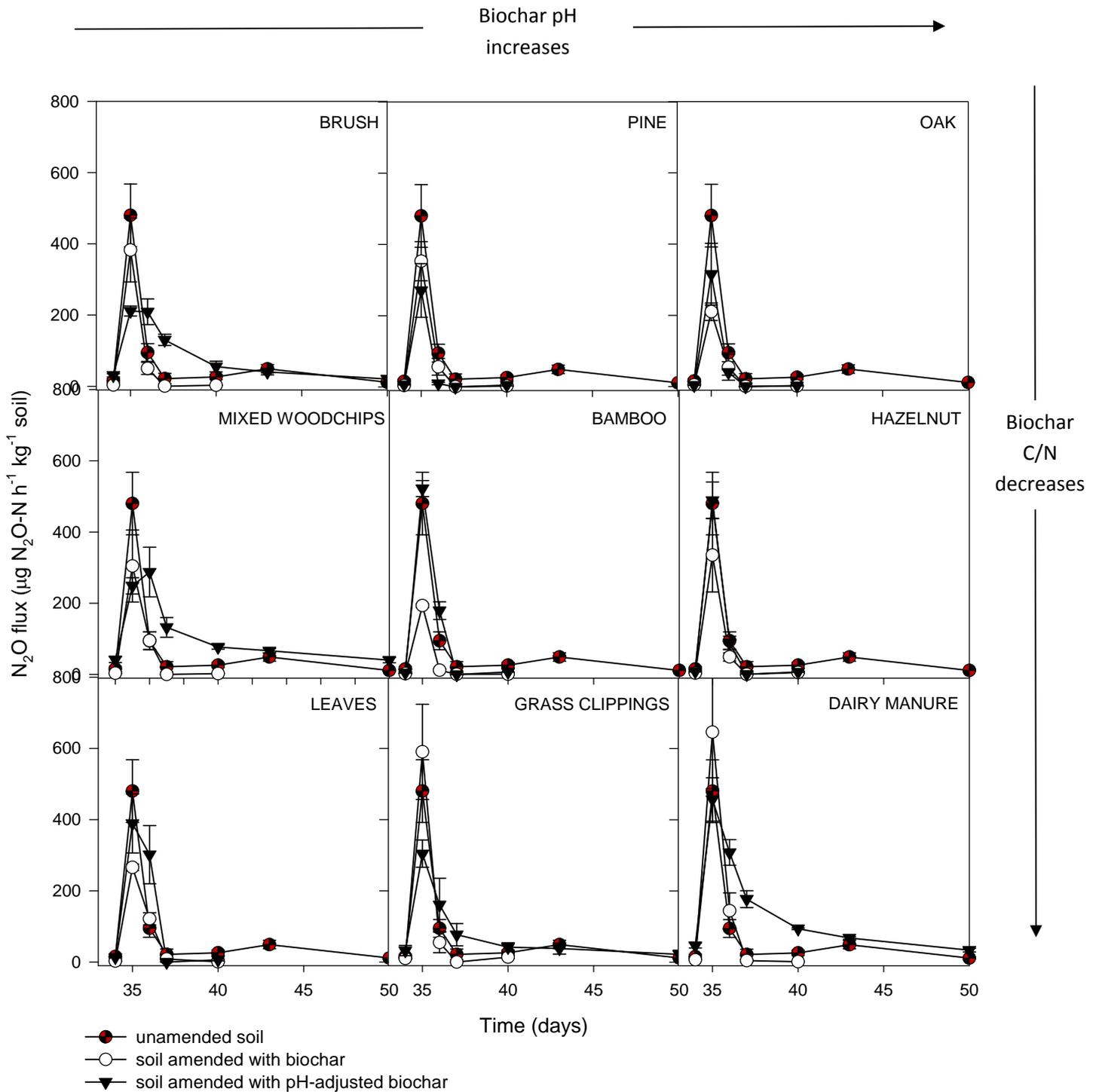


Fig. S4. N_2O fluxes from a muck soil amended with nine different biochars at 2% (dry weight basis). The soil was incubated for 33 days under denitrification conditions (90% WFPS, 30°C). At day 34 all treatments were spiked with a solution of KNO_3 and glucose, at 100 μg N and 1000 μg C per gram of soil, respectively. Graphs are organized from left to right with increasing biochar pH and from top to bottom with decreasing C/N ratios. Error bars represent the standard error of the mean ($n=4$).

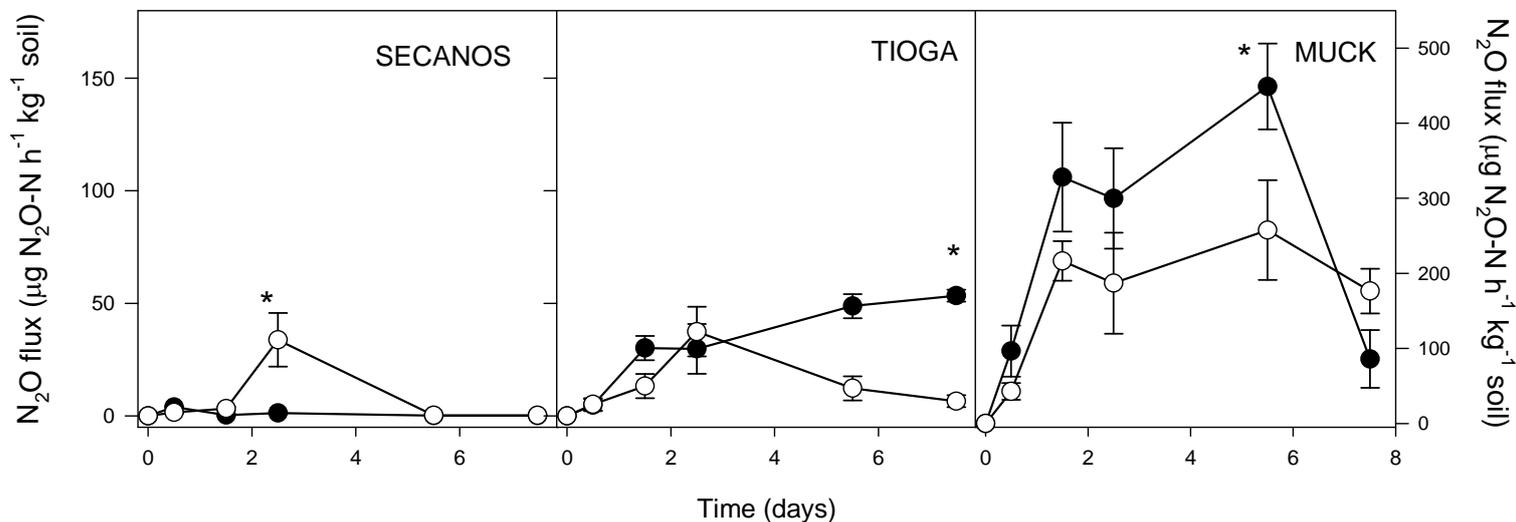


Fig. S5. N₂O fluxes after addition (50mg N kg⁻¹ soil) of KNO₃ (¹⁵N, 99% enrichment) to three agricultural soils pre-incubated under denitrification conditions (90% WFPS, 30°C) for 14 days. Empty dots represent soils amended with biochar (brush, 500°C) at 2% (dry weight basis) and filled dots represent unamended soils (controls). Error bars represent the standard error of the mean (n=4). The asterisk in each graph points at the time when the differences between fluxes from soils amended with biochar in comparison with control soils were greatest (which usually corresponds with the peak of emissions). These samples were selected for analysis of N₂ and N₂O isotopic composition.

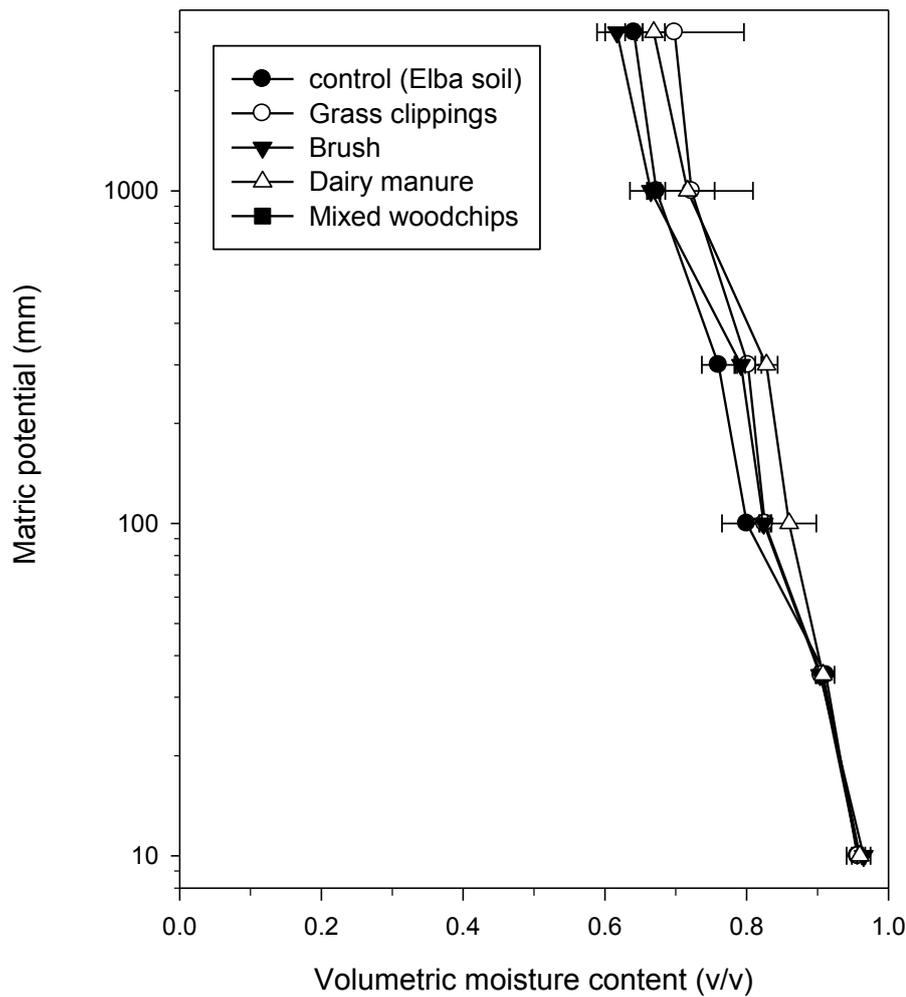


Fig. S6. Water retention curves for the muck soil (control) and different biochar treatments (2% weight) measured using a sand tension table controlled with vacuum pressure regulators according to [1]. Error bars represent the standard deviation of the mean (n=2) for soil without and with a range of different biochars. Repeated-measures ANOVA showed that there was no significant effect of biochar treatment on volumetric moisture content (df =4, P=0.352) and no matric potential × biochar interaction (df (Greenhouse-Geisser correction)=6.5, P =0.224).

1. Topp, G.C. and W. Zebchuk, The determination of soil-water desorption curves for soil cores. Canadian Journal of Soil Science, 1979. **59**(1): p. 19-26.