Supplementary Online Material

DNA Extraction Efficiency from Soil as Affected by Pyrolysis Temperature and Extractable Organic Carbon of High-Ash Biochar

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10 pages, 3 Figures, 4 Tables

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Biochar extraction methods

The extraction method "water floating" that was used to extract biochar particles from soil was modified based on Lin et al. (2012). A 20 g sample of soil biochar mixtures was added to a beaker with 100 ml of sterile water. The mixture was then stirred gently for 2 mins, and the isolated biochar particles in the suspension were collected on a sieve during agitation. In this process, the biochar particles remained on the sieve surface and the soil particles passed through the sieve. The biochar particles were then collected manually using tweezers, gently rinsed with sterile water to remove the residual soil particles, and then stored at -80 °C (The soil particles that were not able to be removed were considered the biochar-sphere). The method of "tweezers" is as follows: Autoclaved tweezers were used to pick the biochars directly from the soils with a magnifying glass under sterile conditions.

Primer specificity test

The 16S rRNA gene in an *A. fischeri* DNA sample and samples without *A. fischeri* DNA added was amplified by polymerase chain reaction (PCR) (Fig. S1). The forward and reverse primers were: Af-F: 5'-GCGGAAACGACTTAACTGAACC-3' and Af-R: 5'-GAAGGTCCCCCTCTTTGGTC-3'. The PCR reaction mixture (20 µl) contained 2.0 µl DNA template, 1.0 µl each primer (10 µM) (IDT, San Jose, USA), 10 µl Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and 6 µl DNase/RNase free water. The following thermal cycling program was used for PCR: 98 °C for 500 s (initial denaturation), 30 cycles at 98 °C for 30 s (denaturation), 60 °C for 45 s (annealing), 72 °C for 30 s (extension), and 72 °C for 600 s (final extension). The V4 region of the 16S rRNA gene in an *A. fischeri* DNA sample and samples without *A. fischeri* DNA added was amplified by PCR (Fig. S1). The sequences of the forward and reverse primers were 515F:5'-GTGCCAGCMGCCGCGGTAA-3' and 806R:5'-GGACTACHVGGGTWTCTAAT-3', respectively. The PCR reaction mixture (20 µl) contained 2.0 µl DNA template, 1.0 µl each primer (10 µM)(IDT, San Jose, USA), 10 µl Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and 6 ul DNase/RNase free water. The following thermal cycling program was used for PCR: 98 °C for 500 s (initial denaturation), 30 cycles at 98 °C for 30 s (denaturation), 55 °C for 45 s (annealing), 72 °C for 30 s (extension), and 72 °C for 600 s (final extension).

Reference

Lin, Y., Munroe, P., Joseph, S., Kimber, S., Van Zwieten, L., 2012. Nanoscale organo-mineral reactions of biochars in ferrosol: an investigation using microscopy. Plant and Soil 357, 369-380.

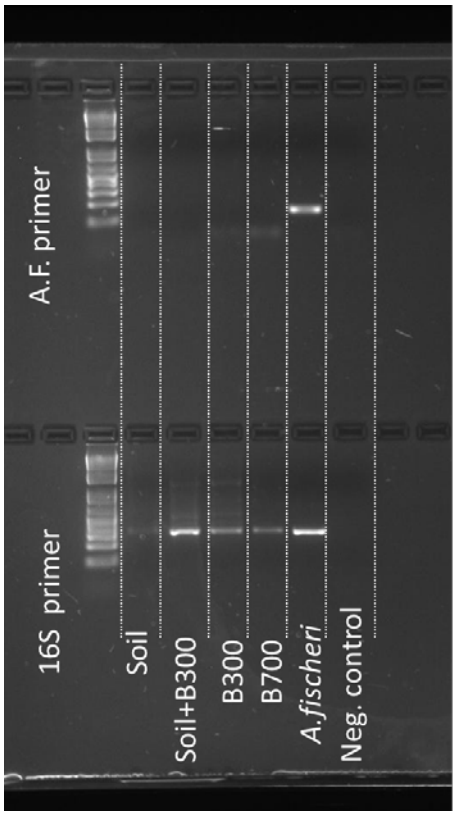

Fig. S1. Specificity test of designed Af-F and Af-R primers targeting the *Aliivibrio fischeri* 16S rRNA genes (top) and universal primers for the V4 region of 16S rRNA gene (bottom) of DNA extracted from soil without amendments in comparison to soil amended with B300, pure B300 or B700, from *A. fischeri*, and a negative control.

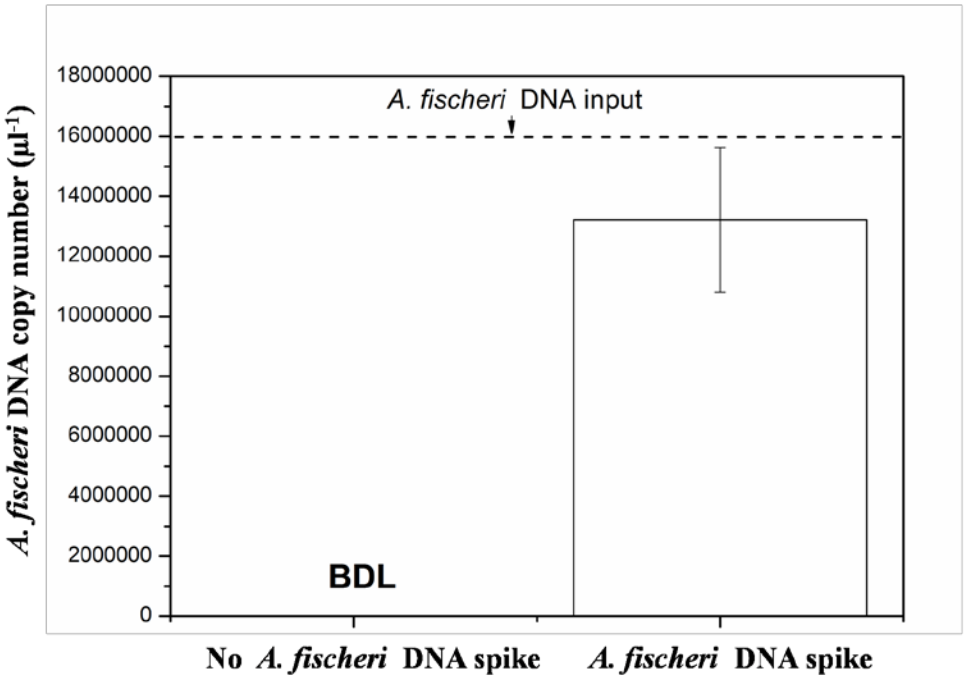

Fig. S2. Spike-recovery test of the *Aliivibrio fischeri* DNA standard in B700 (low DNA extraction efficiency) after extraction, used to rule out potential effects of inhibitors that might have affected the qPCR reaction or organic materials that might have created a false positive. BDL: below detection limit. T-test indicated that the DNA spike was not significanty different compared to the DNA input *(p*>0.05).

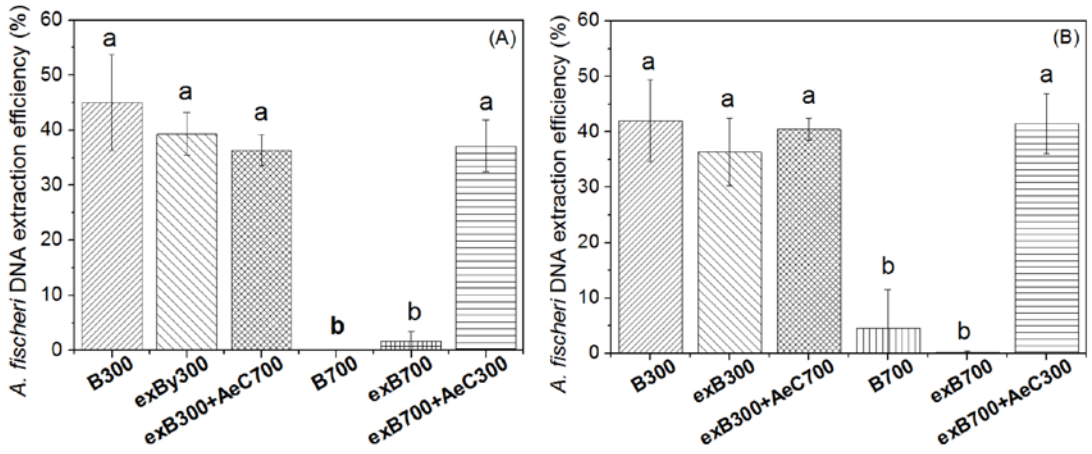

Fig. S3. Extraction efficiency of *Aliivibrio fischeri* from incubated biochars, i.e., isolated by (A) floating on water; or (B) tweezers. Significant differences between biochars were analysed using one-way ANOVA ($p<0.05$). Different lower-case letters represent significant differences at 0.05 probability.

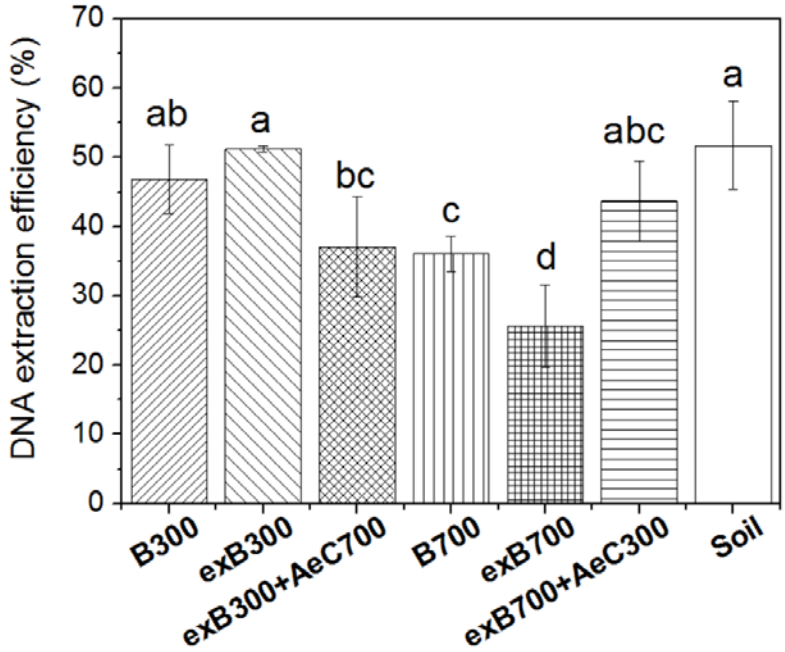

Fig. S4. DNA extraction efficiency of *Aliivibrio fischeri* from biochar-amended soils. Significant differences between soils were analyzed using one-way ANOVA. Different lowercase letters represent significant differences at $p<0.05$.

Table S1. Proximate Analyses (ash content shown in the main manuscript) and Acetone Extraction Rate of B300 and B700. ND = not detected.

Properties	Biochar type	
	B300	B700
Moisture (%)	0.21	0.85
Volatile matter (%)	52.90	6.54
Fixed carbon (%)	15.57	36.61
Acetone extraction rate (%)	15	ND

Table S2. Three-way Analysis of Variance of DNA Extraction Efficiency in Unincubated and Incubated Biochars. The DNA extraction efficiency in incubated biochars was calculated using the average values of both isolation methods of either floating on water or picking with tweezers). ** = $p<0.01$;ns = $p>0.05$.

Factors	F-value	Significance
Acetone extraction category	148	**
Pyrolysis temperature	125	**
Soil incubation effect	11.2	**
Acetone extraction category× Pyrolysis temperature	48.2	**
Acetone extraction category×Soil incubation effect	9.17	**
Pyrolysis temperature ×Soil incubation effect	28.1	**
Acetone extraction category×Pyrolysis temperature ×Soil incubation effect	0.16	ns

Table S3. Effects of Different Extractable Organic Carbon Categories on DNA Extraction Efficiency in Biochars. Different lower-case letters represent significant differences at 0.05 probability.

Acetone extraction category	DNA extraction efficiency (%)
AeC 300	43.4a
AeC 700	14.6b
No AeC	15.4b

Table S4. Significance of Differences in DNA Extraction Efficiency between Unincubated Biochars and Incubated Biochars. A: Unincubated biochars and incubated biochars extracted by floating on water; B: Unincubated biochars and incubated biochars extracted using tweezers. ns = $p>0.05$.

Treatments	A	B
B300	ns	ns
B700	ns	ns
exB300	**$p<0.01$**	**$p<0.05$**
exB700	ns	ns
exB700+AeC300	ns	ns
exB300+AeC700	**$p<0.01$**	**$p<0.01$**