Miscanthus biochar promotes growth of spring barley and shifts bacterial community structures including phosphorus and sulfur mobilizing bacteria

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Supplementary materials and figures for: *Miscanthus* biochar promotes growth of spring barley and shifts bacterial community structures including phosphorus and sulfur mobilizing bacteria.

SM1 - DGGE PCR conditions

PCR conditions for DGGE analysis were as follows: 10X Dream Taq buffer (2 mM $MgCl_2$, final concentration), 0.4 µM of forward and reverse primer, 1M betaine, 0.2 µM dNTPs and 0.5 U of Dream Taq (Fisher Scientific; Waltham, MA) in a final volume of 25 µl. In a GS-2 cycler (G-Storm, UK), a touch-down protocol was executed with an initial denaturation of 3 min (95 °C), 20 cycles of denaturation (94 °C, 45 s), annealing at 65-55 °C (-0.5 °C per cycle, 45 s) and extension (72 °C, 45 s). 20 more cycles at 55°C annealing temperature followed and PCR was completed with an additional 5 min of extension (72 °C).

SM2 - NGS PCR conditions

PCR reactions were conducted in 25 µl with 0.4 µM of each universal primer (16SF and 16SR), 0.2 µM dNTPs, 1X PCR buffer with 1.5 mM $MgCl_2$ and 0.5 U of Robust Taq (Kapa Biosystems; London, UK). A touch-down PCR protocol with 20 cycles of 94 °C denaturation (3 min), 68-58 °C touchdown (-0.5 °C per cycle, 60 s), 72 °C extension (60 s), plus a further 20 cycles with 58 °C annealing temperature was employed.

SM3 - NGS data analysis

Illumina pair-end sequence reads (2 X 250) were merged using flash (Magoč and Salzberg, 2011) and sequences were quality checked using the split libraries script in Qiime (Caporaso et al., 2010). The quality check ran in two phases, first for joining reads with a minimum overlap of 10 bp, and secondly for removing joined reads with anything less than 150 bp and quality of 19 and below. Reads of similar sequence (min. 97 %) were clustered into OTUs and chimeras were removed using the 64-bit version of USEARCH (Vazquez-Baeza et al., 2013). OTUs were then aligned, a phylogenetic tree was constructed and alpha and beta diversity determined within Qiime (Caporaso et al., 2010). Alpha diversity was calculated using the Shannon and Simpson diversity indices and the *Chao 1* richness estimator

(Vazquez-Baeza et al., 2013). Taxonomy was assigned using BLAST against the 16S-specific SILVA database (Version 106).

SM4 - Quantitative PCR conditions

PCR conditions for absolute quantification were as follows: 2X SybrFast master mix (Kapa Biosystems), 0.3 µM of forward and reverse primer and 1M betaine. The PCR protocol was carried out in a Roche 480 II cycler with 4 min initial denaturation (95°C), followed by 40 cycles of 3 s denaturation (95 °C), 20 s annealing (55 °C for *Arthrobacter* and *Brevundimonas*, 60 °C for *Cupriavidus*) and 20 s extension (72 °C). After a final extension step (72°C) a melting curve was conducted. For quantification, qPCR standards were prepared using DNA from bacterial isolates and diluted to 10^6, 10^4, 10^2 and 10^1 copies μl^{-1}.

References:

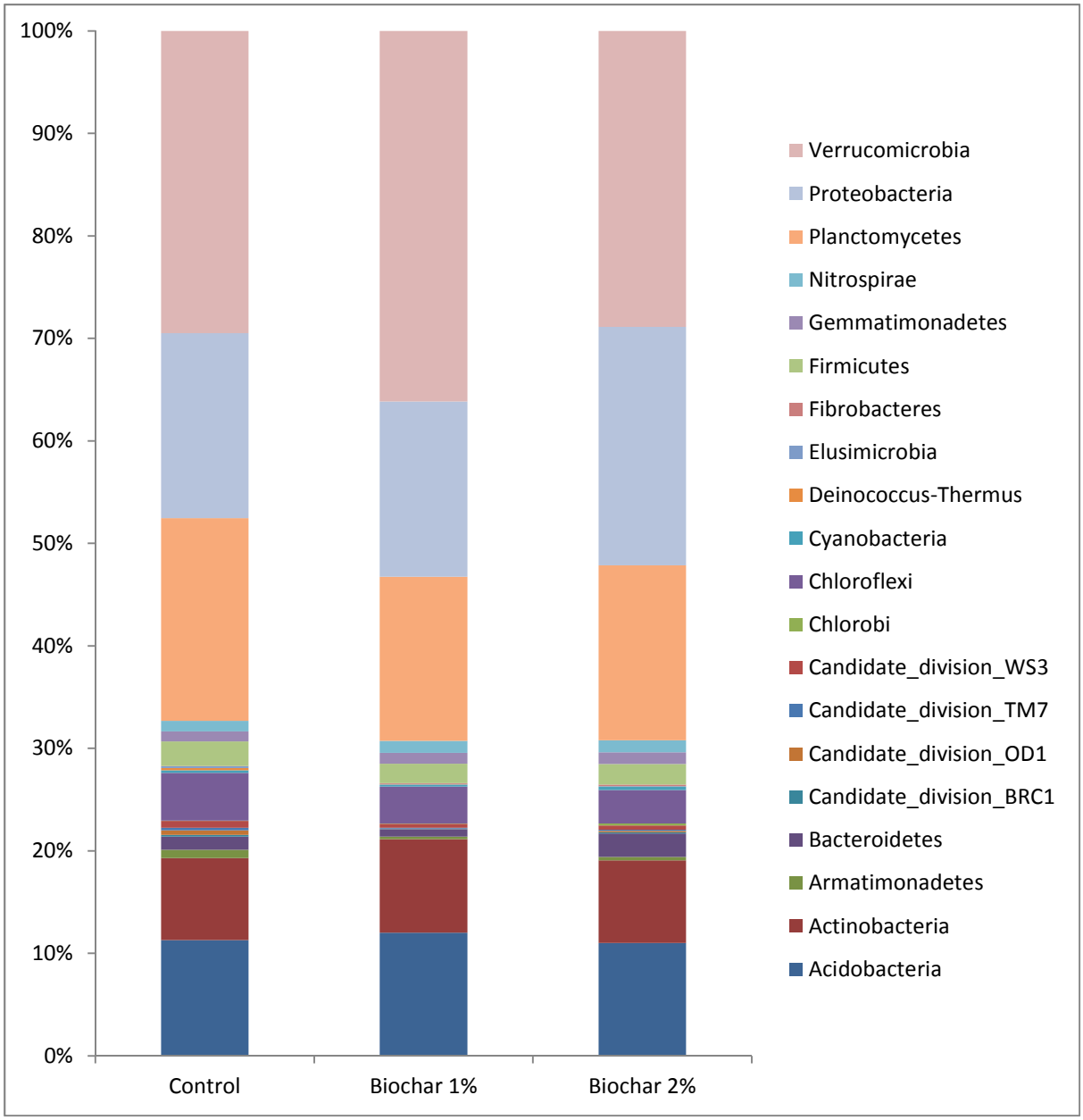
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Meth. 7, 335-336.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. BMC Bioinformatics 27, 2957-2963.

Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., Knight, R., 2013. EMPeror: a tool for visualizing high-throughput microbial community data. GigaScience 2, 16.

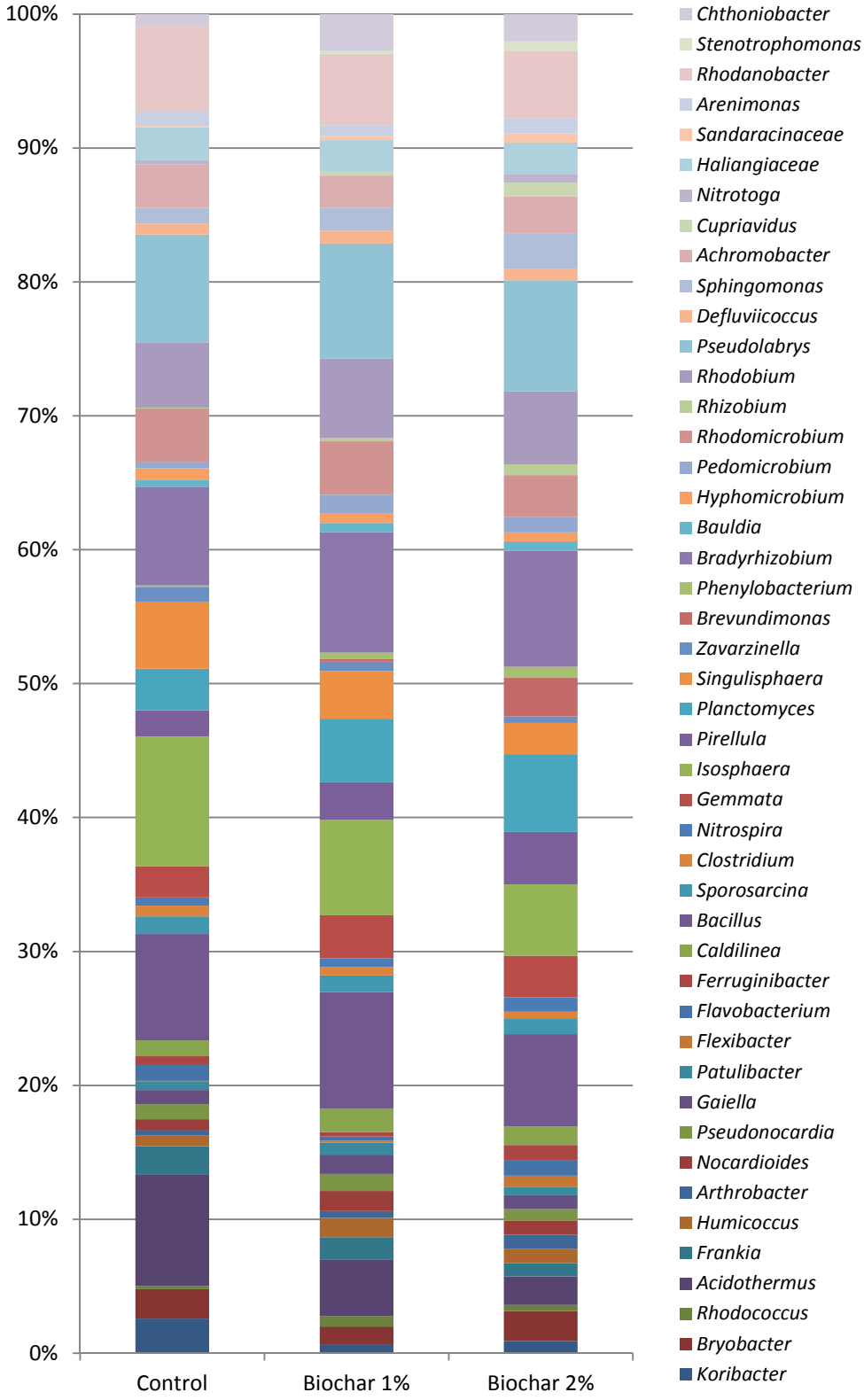
Supplementary figure S1:

Relative abundance [%] of major bacterial phyla (> 0.1 %) in 16S rRNA gene fragment amplicon libraries.



Supplementary figure S2:

Relative abundance [%] of major bacterial genera (> 0.1 % in at least 1 amplicon library) in 16S rRNA gene fragment amplicon libraries.



Supplementary figure S3:

Normalised S K-Edge XANES spectra from biochar before incubation (blue line), *Miscanthus* grass feedstock (red line) and soil (green line, before spring barley growth experiment).