Biochar alteration of the sorption of substrates and products in soil enzyme assays: Supplementary material

Authors: Mark A. Swaine*, Rachel Obrike**, Joanna M. Clark, Liz J Shaw†

Soil Research Centre, Department of Geography and Environmental Science, University of Reading, Reading, RG6 6DW, UK

†Corresponding author: Liz J Shaw; e-mail address: e.j.shaw@reading.ac.uk; Tel.: +44 (0)118 3786971; Fax: +44 (0)118 3786660

*Present address: SRUC, Crop and Soil Systems Research Group, West Mains Road, Edinburgh, EH9 3JG, UK.

**Present address: Reading Scientific Services Ltd., Reading Science Centre, University of Reading, Reading, RG6 6LA, UK.

Supplementary Material

Additional methodological details

Conversion of p-nitrophenyl phosphate (pNPP) to p-nitrophenol (pNP) using alkaline bovine phosphatase.

To quantify pNPP concentrations in experiments examining biochar effects on the concentration of pNPP under enzyme assay incubation conditions, alkaline bovine phosphatase (Sigma-Aldrich) was used to convert pNPP to pNP. The concentration of the yellow pNP chromophore produced could be quantified using a spectrophotometer (400 nm) and the effect of biochar on pNPP concentration calculated from pNP concentration data with the knowledge that 1 mole of pNPP is hydrolysed to 1 mole of pNP. After removal of soil from pNPP incubations by centrifugation (see section '*Biochar effects on concentrations of assay substrates and products'*, main text) supernatant (0.3 ml) was added to 2.7 ml Diethanolamine (DEA) buffer (1 M in 0.5 mM $MgCl_2$; pH 9.8) to which 0.1 ml bovine phosphatase (0.1 unit ml^{-1}) was added. Samples were incubated (37 ^{o}C) for 16 hours, a time shown in preliminary experiments (Fig. S1) to be sufficient to allow complete hydrolysis of the maximum theoretical amount of pNPP possible in the enzyme mix (0.3 ml x 23 µmol ml^{-1} = 6.9 µmol). After incubation, the concentration of pNP in 1/50 diluted samples was determined spectrophotometrically at 400 nm (Cecil CE 292 digital ultraviolet spectrophotometer series 2) against a 1/50 diluted blank of DEA buffer. Enzyme-only and pNPP-only controls were included in the incubations.

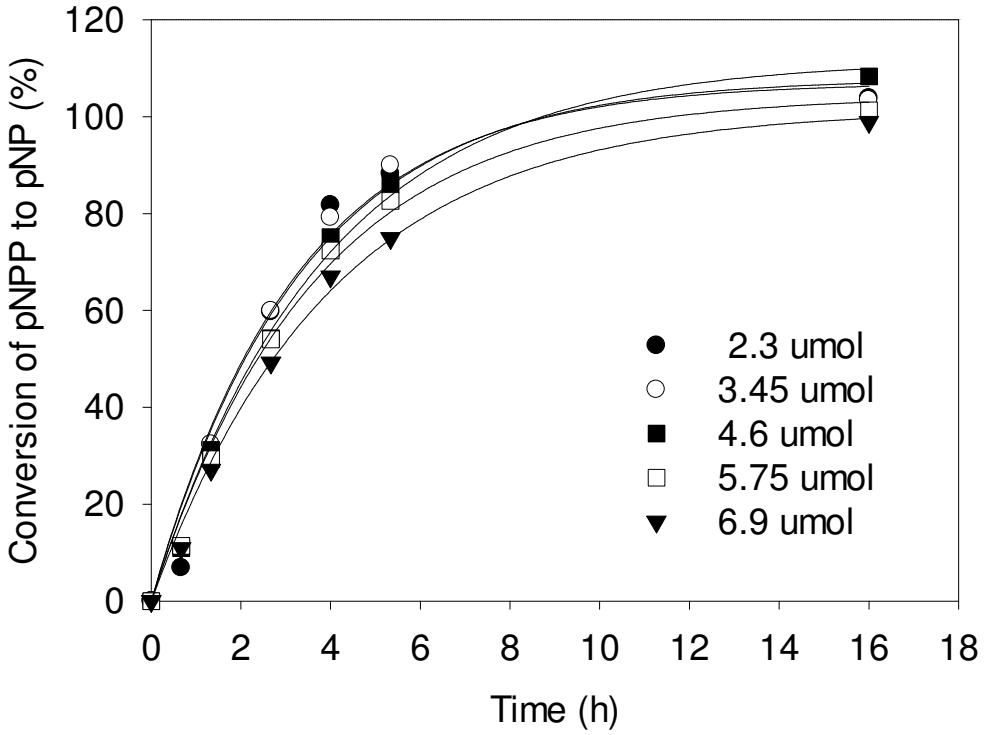

Figure S1. Kinetics of conversion of differing amounts (2.3 µmoles to 6.9 µmoles) of p-nitrophenyl phosphate (pNPP) to p-nitrophenol (pNP) using alkaline bovine phosphatase as quantified by spectrophotometric detection (400 nm) of pNP production. Production of pNP was negligible in enzyme-only and pNPP-only controls; control data are not plotted for clarity.