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Supplementary Material

Biochar increases soil enzyme activities in two contrasting pastoral soils under different grazing management

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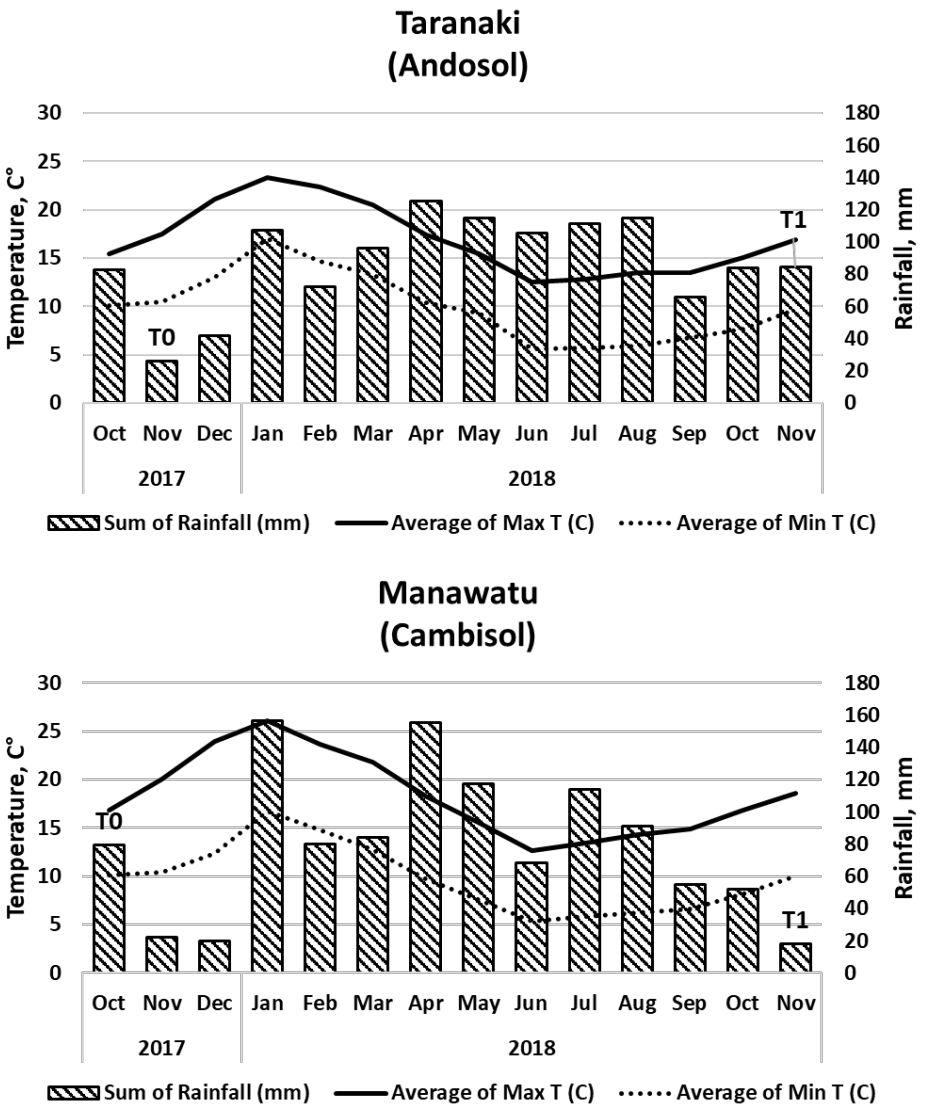

Figure S1. Average monthly temperatures (max and min) and precipitation during the time of the experiment (source: Meteorological Services of New Zealand Limited). T0, "initial" soil before the start of the experiment, T1, sampling at 12 months.

Soil enzyme analysis methods

Cellulase (EC 3.2.1.4) activity in the soil was determined by the Pancholy and Rice method (Pancholy and Rice, 1973). For this, 0.5 g of air-dry soil (<0.25 mm) was pre-treated for 15 min with 0.05 mL toluene and further incubated with 1 mL of sodium acetate buffer (pH 5.9) and 1mL carboxymethylcellulose (1%) at 30 °C for 24 h. After incubation, 8 mL DI H_2O was added and mixed well. The suspension was then centrifuged for 10 min at 5,000g. The concentration of reducing sugars in 1 mL of supernatant was measured by Somogyi-Nelson method (Deng and Tabatabai, 1994) with a spectrophotometer at 520 nm, with glucose as a standard for making the calibration curve.

Soil urease (EC 3.5.1.5) activity was determined by the Shcherbakova method (Shcherbakova, 1983). For this, 0.25 g of air-dry soil (<0.25 mm) was incubated with 0.3 M urea in 0.2 M phosphate buffer (pH 6.5) with 0.02 mL of toluene at 37 ^{0}C for 4 h. After incubation, the reaction was stopped by adding 20% trichloroacetic acid and 5 mL of 1 M KCl. The suspension was centrifuged for 10 min at 5,000g. The 0.2 mL of supernatant was dissolved in 4.4 mL of DI H_2O, and 0.2 mL of 50% potassium sodium tartrate (Seignette reagent) and 0.2 mL potassium tetraiodomercurate (Nessler reagent) were added. The concentration of released NH_4^+-N was measured with a spectrophotometer at 400 nm. Ammonium chloride standard solutions were used the make the calibration curve.

Alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) phosphatase activities in the soil were determined by the Tabatabai and Bremner method (Tabatabai and Bremner, 1969). A 0.1 g sample of air-dry soil (<0.25 mm) was incubated with 2 mL of modified universal buffer (pH 6.5 for the acid phosphatase and 11.0 for the alkaline phosphatase) and 0.5 mL of 0.115 M p-nitrophenyl phosphate solution for 1 hour at 37 ^{0}C. After incubation, 0.5 mL of 0.5 M $CaCl_2$ and 2 mL of 0.5 M NaOH were added, and the mixture was centrifuged for 10 min at 5,000g. The concentration of released p-nitrophenyl was measured with a spectrophotometer at 400 nm. Calibration curve was made with standard solutions of p-nitrophenol.

Soil nitrate reductase (EC 1.7.99.4) activity was determined by Kandeler method (Schinner et al., 1996). A 1 g sample of air-dry soil (<0.25 mm) was incubated with 25 M KNO_3 solution and 1 mL water with added 0.9 mM 2,4-dinitrophenol solution as inhibitor of nitrite reductase at 25 ^{0}C for 24 hours. After incubation 1.5 mL of 4 M potassium chloride was added. The suspension was centrifuged for 10 min at 5,000g. Then, 2.5 mL of the supernatant was mixed with 1.5 mL of ammonium chloride buffer (0.19 M, pH 8.5) and 1 mL of colour reagent (sulfanilamide and 0.1 g of N-(1-naphthyl) ethylenediamine dihydrochloride). The concentration

of released NO_2^- was measured with a spectrophotometer at 520 nm. Sodium nitrite standard solutions were used for making the calibration curve.

Peroxidase (EC 1.11.1.7) activity was determined by the Karyagina–Mikhailovskaya method (Khaziev, 2005). The 0.5 g air-dried soil (< 0.25 mm) was incubated with 25 mL of freshly prepared 0.1 M hydroquinone and 0.25 mL of 0.5% hydrogen peroxide. The mixture was thoroughly mixed and kept at 30°C in a thermostat for 30 min. The reaction was stopped by the addition of 10 mL of 96% ethanol, and the reaction mixture was centrifuged for 10 min at 5,000g. The content of the formed 1,4-benzoquinone was measured with a spectrophotometer at 450 nm; 1,4-benzoquinone standard solutions were used for making the calibration curve.

Dehydrogenase (EC 1.1.1.x) activity was measured by the Thaimann method (Alef, 1995). The 0.8 g of air-dried soil (<0.25 mm) was incubated with 1.8 ml triphenyl tetrazolium chloride solution (7.5 mg/ml) in Tris-HCl buffer (pH 7.6) for 24 h at 30°C. The reaction was stopped by the addition of 5 ml acetone and kept at room temperature for 2 h in the dark. Then the solution was centrifuged for 10 min at 5,000g. The content of the formed product was measured with a spectrophotometer at 546 nm. Triphenyl formazan standard solutions were used for making the calibration curve.

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