Ren H., Chen X., Qin X., Zhang S., Lv C., Zhou J., Chen H. (2024). Bacterial fertilizer and filtered sludge enhance soil fertility and plant growth in a eucalyptus plantation. Silva Fennica vol. 58 no. 5 article id 24042. https://doi.org/10.14214/sf.24042

Supplementary file S4

Methods

Sucrose. Sucrose enzyme can hydrolyze sucrose into glucose and fructose. (1) To prepare a standard glucose solution, 500 mg of glucose was oven-dried in an oven at 50 °C until reaching a constant weight. Then glucose was dissolved in 100 ml of benzoic acid solution to create a 5 mg/ml standard glucose solution. A standard curve is generated through a microplate reader at the wavelength of 508 nm; (2) Dried soil samples (5 g) are placed in a dry conical flask. We then added 5 ml of phosphate buffer with a pH of 5.5, 15 ml of an 8% sucrose solution and 5 drops of toluene. After thorough mixing, the samples are incubated at 37°C for 24 hours and then filtered. 1 ml of the filtrate is taken and mixed with 3 ml of 3,5-dinitrosalicylic acid, then heated in a water bath for 10 minutes. The solution is rinsed under tap water until an orange-yellow color develops. Subsequently, the volume is adjusted to 50 ml with distilled water, and the absorbance of the mixed solution is measured using a microplate reader at 508 nm. The activity of the sucrose enzyme is expressed as milligrams of glucose per gram of soil after 24 hours.

Urease. (1) We first took 0.4717g of ammonium sulfate and dissolve it in 1000ml of distilled water to prepare a nitrogen standard solution with a concentration of 0.1mg/ml. After diluting the standard solution by a factor of ten, we took 1, 3, 5, 7, 9, 11, and 13ml for later use. Next, we add 3ml of sodium hypochlorite and 4ml of sodium phenolate and mixed them thoroughly. After 20 minutes, we adjust the volume to 50ml with distilled water and conducted the colorimetric measurement at a wavelength of 578nm. (2) We took 5g of air-dried soil sample and then add 1ml of toluene. After 15 minutes, we add 20ml of a citrate buffer with a pH of 6.7 and 10ml of a 10% urea solution, mixing them thoroughly. After incubating at 37℃ for 24 hours, we filtered the mixture. We mixed 3ml of the filtrate, add 3ml of sodium hypochlorite, and 4ml of sodium phenolate. After development of color, we adjust the volume to 50ml with distilled water and conducted the colorimetric measurement at a wavelength of 578nm after 1 hour.

CAT. We took a 2g air-dried soil sample and placed it in a 100ml Erlenmeyer flask. Then, we added 5ml of 3% hydrogen peroxide solution and 40ml of distilled water, mixing thoroughly before shaking it on an oscillator for 30 minutes. After this, we added 5ml of 3mol/l sulfuric acid to ensure a complete reaction, fully eliminating any errors caused by residual hydrogen peroxide that may affect the experiment. Finally, we extracted 25ml of the filtered solution and titrated it with 0.1mol/l potassium permanganate until reaching a pink endpoint. The consumption of potassium permanganate was recorded to indicate CAT enzyme activity.