

#### Supplementary. Text S1.

Chlorophyll a (CHLa), chlorophyll b (CHLb), and carotenoid contents were determined in plant leaves (50 mg). Samples were ground in a BIOGEN mixer mill (model Mill Mix 20) with 80% acetone, and the absorbance of the extracts was measured at 663.2, 646.8 and 470.0 nm using a Beckman DU530 spectrophotometer. The equations described by Lichtenthaler [58] were used to determine the concentration of photosynthetic pigments, expressed in mg/g of plant (fresh weight, FW).

Reactive oxygen species (ROS) levels were determined by incubation with a 50  $\mu$ M solution of 2',7'-dichlorodihydrofluorescein diacetate ( $H_2DCF$ -DA). Leaf disks (6 mm diameter) from three plants per replicate were incubated for 30 min, homogenised in 500  $\mu$ l of 10% ethanol in water using a BIOGEN Mixer Mill, centrifuged and the fluorescence of the supernatant was measured (excitation 485 nm; emission 535 nm). Fluorescence values were normalized to protein levels and expressed as fluorescence per mg of protein.

Malondialdehyde (MDA) content was measured in leaf pieces (0.10 g), which were ground in 0.665 mL of 10% trichloroacetic acid (TCA) in a BIOGEN Mixer Mill and the MDA in the supernatant was measured by reaction with 0.5 % of thiobarbituric acid in TCA (20%). The MDA concentration was determined by subtracting the absorbance at 600 nm from the absorbance at 532 nm (extinction coefficient 155  $mM^{-1} cm^{-1}$ ). Values were expressed as nmol per g of plant (FW).

Enzymes and proteins were determined in leaves (0.15 g), which were homogenized with 0.45 mL of a pH 7.8 solution of 50 mM sodium phosphate buffer, 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone using a BIOGEN Mixer Mill. After centrifugation (13,000 rpm, 10 min, 4°C), the enzyme activities and protein content of the supernatant were determined. Catalase (CAT) activity was determined by measuring the consumption of  $H_2O_2$  at 240 nm. One unit of CAT activity corresponded to 1  $\mu$ mol of  $H_2O_2$  consumed per minute per mg of protein using an extinction coefficient of 40  $M^{-1} cm^{-1}$ . Ascorbate peroxidase (APX) and guaiacol peroxidase (GPOD) activities were measured in 96-well microplates using a GENios microplate spectrofluorometer (TECAN) at 290 nm and 470 nm, respectively. One unit of enzymatic activity was defined as the amount of enzyme that caused a change of 0.1 absorbance units per minute per mg of protein. The protein content in each extract was determined by the method developed by Bradford [59]. Values were expressed as mg protein per g of plant (FW)

#### Supplementary. Text S2.

Dehydrogenase (DH) activity was measured in samples of alfalfa-enriched fresh soil (1 g, DW). Soil samples were incubated with 0.2 mL of 2,3,5-triphenyltetrazolium chloride 3% (w/v) and 0.5 mL of glucose 0.5% (w/v) in deionized water at 27°C for 24 h. The reaction product (triphenylformazan (TPF)) was extracted with 2.5 mL of methanol. Absorbance was measured at 490 nm in 96-well microplates in a TECAN). DH activity was calculated as  $\mu$ g of TPF produced per g of soil during 24 h.

Phosphatase activity was measured by incubating 1 g (DW) of fresh soil with 3.2 mL of pH 6.5 buffer (50 mL of 1M NaOH, 1.21 g of tris(hydroxymethyl)aminomethane chlorhydrate (TRIS), 1.16 g of maleic acid, 1.4 g of citric acid and 0.63 g of boric acid in 500 mL of water) for 30 min at 27°C. Samples were then incubated for 2 h in the dark at 27°C with 80  $\mu$ L of 0.01 M methylumbelliferyl phosphate in buffer. The fluorescence of 4-methylumbelliferone (MU) formed was measured in a TECAN (excitation 320 nm; emission 465 nm). Activities were calculated as  $\mu$ g of MU generated per g soil.