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RESEARCH ARTICLE

Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics

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Abstract
In tropical no-till systems, applying lime (L) and phosphogypsum (PG) on the soil surface may be a potential strategy for reducing soil acidification and improving soybean root growth, thereby enhancing plant nutrition and physiological responses and, in turn, crop resistance to dry spells. This study evaluated the impact of long-term (17 years) surface soil amendment on soil fertility and soybean root development, nutrition, gas exchange, carbon and antioxidant enzyme activity, and grain yield in a tropical region subject to dry spells. The treatments consisted of the following long-term soil amendments: control (no soil amendment); L alone; PG alone; and L + PG (LPG). Liming, especially when combined with PG, improved soil fertility, as evidenced by increases in pH and P, Ca^{2+}, and Mg^{2+} levels throughout the soil profile, but reduced Al^{3+} and micronutrients (Fe, Mn, Cu, and Zn). The improvements in soil fertility were associated with increased root development throughout the profile. Long-term application of LPG reduced the negative impacts of dry spells on pigment concentrations, gas exchange, Rubisco and sucrose synthase activities and antioxidant metabolism, and increased soybean grain yield. Our results reveal that long-term application of LPG is an important approach for increasing the vertical movement of cationic bases and roots in no-till systems to improve soybean nutrition. Long-term amendment with LPG enhanced both carbon and antioxidant metabolism in soybean plants, resulting in higher soybean grain yield, despite the predisposition of this tropical region to dry spells.

Keywords *Glycine max* (L.) Merrill · Soil amendments · Soil acidity · Rubisco · Sucrose synthase · Oxidative stress

1 Introduction

Soybean (*Glycine max* (L.) Merrill) is important both as an oil–protein seed crop for livestock and aquaculture feeds and as a biofuel feedstock (Sugiyama et al. 2014). Soybean is unique among crops in that it can supply protein equal in quality to that of animal sources, making it an excellent protein source for the human diet (Hartman et al. 2011; Sugiyama et al. 2014). Demand for soybean and its derivatives has increased in the last decade, challenging the reliability of supply, stock levels, and competitive pricing (Hartman et al. 2011). The anticipated growth of the global population to approximately 10 billion people by 2050 (Desa 2015) and the effects of climate change (Gupta et al. 2020) have heightened the need for sustainable and highly efficient agricultural practices to guarantee global food security (Rellán-Álvarez et al. 2016).

Among the numerous crop system challenges faced by growers, low soil quality and reduced nutrient availability

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associated with water restriction (due to unpredictable weather or inappropriate management practices) are the main factors responsible for losses in agricultural production, particularly in tropical regions (Rellán-Álvarez et al. 2016; Costa et al. 2018). The detrimental effects of climate change may potentially be alleviated by adopting appropriate soil management practices, particularly soil chemical correction, which is increasingly necessary to improve the acquisition of soil resources by plants (Lal 2009). Soil amendments such as lime (L), a corrective agent for soil acidity with low solubility (Crusciol et al. 2019), and phosphogypsum (PG), a subsurface conditioning agent with moderate solubility (Zoca and Penn 2017), can improve soil quality (Bossolani et al. 2020). In no-tillage systems (NTS), L is applied superficially, which may limit its effectiveness due to its poor solubility, whereas PG has greater mobility in the soil profile but does not correct soil acidity (Zoca and Penn 2017). Thus, combining L and PG can be an alternative for improving the plant growth environment in tropical soils (Bossolani et al. 2020).

In addition to reducing soil acidity, soil amendment with L + PG neutralizes elements toxic to plants, such as aluminum (Al^{3+}) and manganese (Mn). Aluminum inhibits root growth, resulting in reduced water and nutrient uptake as well as plant growth (Reis et al. 2018) and thus reducing Al^{3+} toxicity promotes appropriate conditions for crop root development, particularly in deep soil layers (Costa et al. 2018). Moreover, amendment with L + PG supplies plants with nutrients such as calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorus (P), and sulfate (SO_4^{2-}-sulfur) (Crusciol et al. 2019; Bossolani et al. 2020). Increasing Ca^{2+} availability in the subsurface promotes hormone signaling and root growth (Zoca and Penn 2017), and root development into deeper soil layers is crucial for avoiding plant injury under water-deficit conditions (Costa et al. 2018). In turn, deeper roots increase soil nutrient availability (e.g., Ca^{2+}, Mg^{2+}, P, and SO_4^{2-}-sulfur), resulting in improved plant nutrition and increased photosynthetic activity. Adequate soil nutrient availability promotes photosynthetic pigment formation, energy production, activation of enzymes involved in CO_2 fixation, photoassimilation partitioning for plant development, and the yield index (Gómez et al. 2019). Well-nourished plants are more tolerant of environmental abiotic stresses and have strong antioxidant scavenging systems, which reduces cellular damage caused by reactive oxygen species (ROS) (Santos et al. 2017).

This study focuses on the impact of soil chemical attributes on the physiological and yield responses of soybean to long-term application of soil amendments (Fig. 1). According to Cusser et al. (2020), management recommendations based on short-term studies are generally contradictory and not representative of the system's equilibrium behavior, and therefore, long-term studies are necessary to represent the reality of management within an ecosystem. We investigated the impact of L and PG applied alone or in combination on soil fertility and root growth, especially in deeper soil layers. Simultaneously, the effects of these changes on the physiological status of soybean plants were examined under field conditions to understand how photosynthetic/carbon and antioxidant metabolism are altered by the use of soil amendments and converted into crop yield. To adequately represent the real field conditions of soybean cultivated in rainfed areas, we evaluated soybean plants cultivated under tropical field conditions (i.e., without water supplementation and in a region prone to agroclimatic risks with dry spells during the crop cycle) during the second and third growing seasons after the last soil amendment applications (referred to as the first and second growing seasons hereafter).

2 Material and methods

2.1 Site description and experimental design

This study carried out over two growing seasons is part of a long-term field experiment (registered on the GLTEN Metadata Portal; https://www.glten.org/experiments/62) conducted in Botucatu in southeastern São Paulo State, Brazil (22° 83′ 3″ S, 48° 42′ 64″ W, elevation 765 m above sea level). The soil was classified as a sandy clay–textured Typic Haplorthox (USDA 2014), which corresponds to Oxisols (Jahn et al. 2006). The chemical and textural properties of the soil at 0.0–0.2 m depth, determined prior to installing the experiment in 2002 (Kiehl 1979; van Raij et al. 2001), were as follows: soil pH ($CaCl_2$), 4.2; soil organic matter (SOM), 21 g kg^{-1}; phosphorus (P_{resin}), 9.2 mg kg^{-1}; potassium (K^+), 1.2 $mmol_c$ kg^{-1}; exchangeable calcium (Ca^{2+}), 14 $mmol_c$ kg^{-1}; exchangeable magnesium (Mg^{2+}), 5 $mmol_c$ kg^{-1}; total acidity at pH 7 (H + Al), 37 $mmol_c$ kg^{-1}; cation exchange capacity (CEC), 57.2 $mmol_c$ kg^{-1}; and base saturation (BS), 35%. The soil particle size distribution (inorganic matrix) in the 0.0–0.2-m layer was 545 g kg^{-1} of clay, 108 g kg^{-1} of silt and 347 g kg^{-1} of sand. The clay content at 0.2–0.4 m was 360 g kg^{-1}. The climate is Cwa, which corresponds to a mesothermic type with dry winters and hot summers according to the Köppen–Geiger climate classification system (Alvares et al. 2013). The long-term (1956–2019) maximum and minimum mean temperatures during the soybean growing season were 26.1 °C and 15.3 °C, and the average annual precipitation was 1360 mm (Unicamp 2020).

The experiment was designed in randomized complete blocks (RCB) with four replications. The size of each plot was 56.7 m^2 (6.3 m × 9.0 m). The following treatments were applied: (i) control (no soil amendment); (ii) lime (L); (iii) phosphogypsum (PG); and (iv) lime + phosphogypsum (LPG). From 2002 to 2019, crop fertilization was performed in the same way in each treatment, and only the soil amendments specified in the treatment were applied. The anhydrous

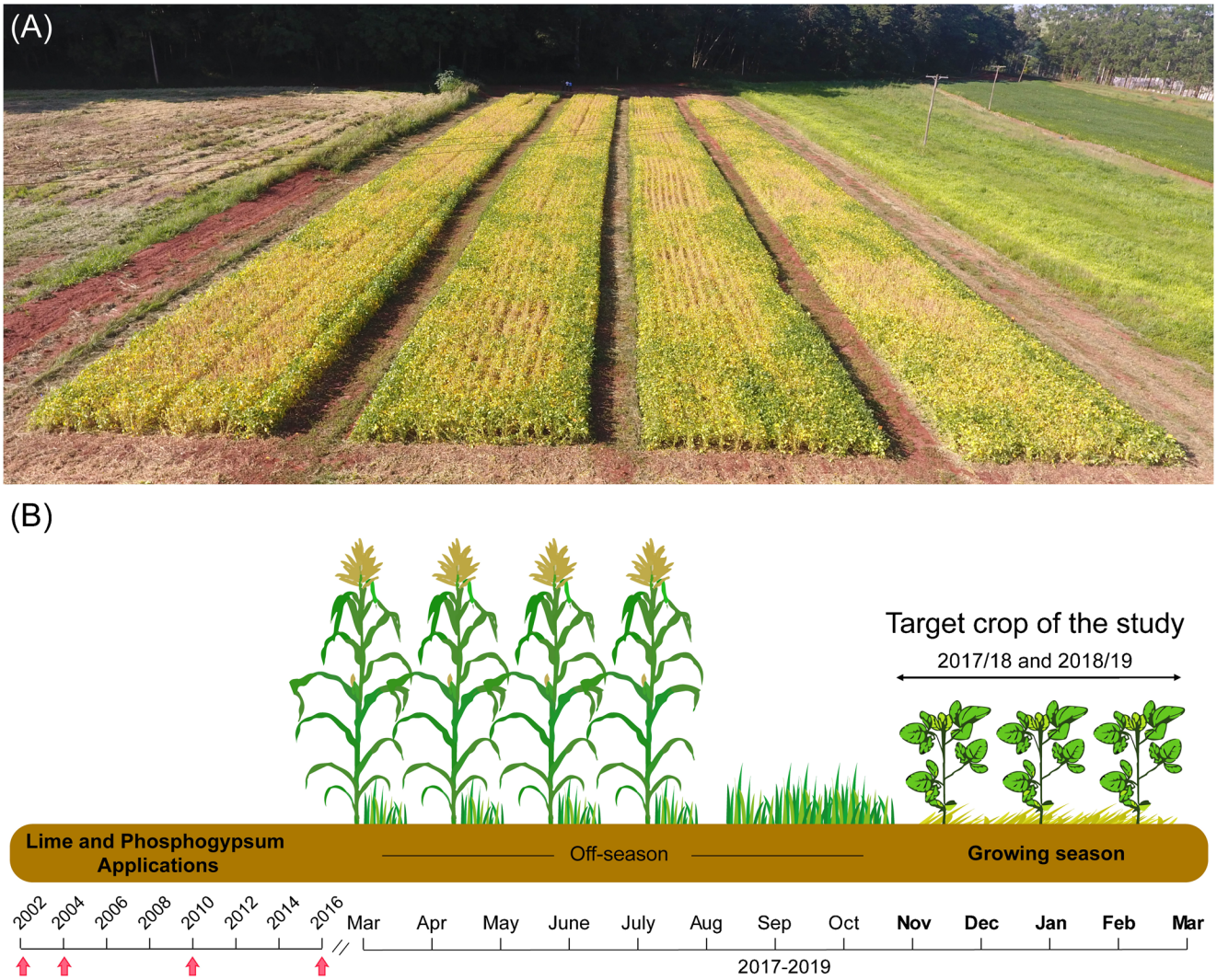


Fig. 1 (**A**) Aerial view of the study area, which is part of a long-term experiment started in 2002. (**B**) Schematic representation of the cropping system, including the chronological sequences of the soil amendment applications and crops grown during the agricultural year. Red arrows indicate when soil amendment applications occurred. Photograph by J.W. Bossolani.

carbonate mineral ($CaMg(CO_3)_2$) containing 166 g kg^{-1} Ca and 105 g kg^{-1} Mg, and the PG contained 200 g kg^{-1} Ca, 150 g kg^{-1} S, < 1g kg^{-1} P, and < 1g kg^{-1} F. At the beginning of the experiment (2002), the L rate was defined according to soil chemical analysis at 0.0–0.2 m depth, and 2.7 Mg ha^{-1} of L was applied with the aim of raising the base saturation (BS) to 70% (van Raij et al. 1997). The PG rate of 2.1 Mg ha^{-1} was calculated according to van Raij et al. (1997) by multiplying the clay content (0.2–0.4 m depth) by a factor 6.

The soil amendments were reapplied when the BS in the L treatment fell to below 50%, which was verified annually. When L reapplication was necessary, PG was also reapplied (2.1 Mg ha^{-1}). In total, the soil amendments were applied four times, including in 2002, 2004, and 2010 (2.0 Mg ha^{-1} of L and 2.1 Mg ha^{-1} of PG). For the fourth application, which occurred in October 2016, updates to the methods used in Brazil led to changes in the calculation of the L and PG rates. Specifically, the L rate calculation method was revised to consider the 0.0–0.4-m layer. The 0.0–0.4-m layer was chosen as the new criterion based on results obtained by Carmeis Filho et al. (2017) for the same experimental area. Carmeis Filho et al. (2017) found that the classic liming recommendation based on the 0.0–0.2-m layer represents an underestimate for stable cropping systems under no-till with crop rotation and high input of crop residues throughout the agricultural year. Consequently, the rate required to raise the BS to 70% was 13 Mg ha^{-1} of L in 2016. The PG recommendation was also revised following the methodology proposed by Caires and Guimarães (2018). This new recommendation, which was intended to increase Ca^{2+} saturation in the effective cation exchange capacity to 60% in the 0.2–0.4-m soil layer, resulted in a rate of 10 Mg ha^{-1} of PG in 2016. After each of the soil amendment applications in 2002, 2004, 2010, and 2016, a micronutrient-based fertilizer was applied over the total area

at rates of 3 kg ha^{-1} of B + 1 kg ha^{-1} of Cu + 1 kg ha^{-1} of Mn + 10 kg ha^{-1} of Zn + 0.2 kg ha^{-1} of Mo in order to avoid unavailability of micronutrients due to the increase in pH by liming.

The current study reports the results for the soybean crops grown in the second and third years after the last soil amendment reapplication (referred to as the first and second growing seasons hereafter). During the entire period of the experiment (2002–2019), several crops were grown. Details of these previous crops and the applications of L and PG are shown in Table S1. With the exception of the first application of L and PG in 2002, when the no-tillage system was initiated, all soil amendments were surface applied.

2.2 Crop sowing and establishment

Soybean (cultivar TMG 7062 IPRO) was sown in November 2017 and 2018 and fertilized with 300 kg ha^{-1} of 04–20–20 (N–P_2O_5–K_2O). The seeds were treated with fungicides (carboxin + thiram at 100 g + 100 g a.i. 100 kg^{-1} of seeds) prior to inoculation and sowing. Seed inoculation was performed 1 h before sowing by evenly coating the seeds with an appropriate amount of inoculant containing strains SEMIA 5079 (*Bradyrhizobium japonicum*) and SEMIA 5080 (*Bradyrhizobium diazoefficiens*). Phytosanitary treatments were carried out according to the needs of and recommendations for the soybean crop (Embrapa 2020).

2.3 Meteorological data

The tendency for short dry spells in southeastern Brazil during the monsoon season (from October to April) was comprehensively established by Cunningham (2020) based on an analysis of a historical series of 37 austral summer seasons (from 1979 to 2016). During the experimental period, meteorological data (rainfall, solar radiation, wind speed, relative humidity, and maximum and minimum temperatures) were measured by an automatic meteorology station installed near the experimental area. The evapotranspiration reference (ET_0) was calculated based on the Penman–Monteith methodology (Allen et al. 1998). Soybean evapotranspiration (ETc) was calculated using the crop coefficient (Kc) for each phenological stage (Allen et al. 1998). Rainfall data were used to monitor the climatological water balance, which was calculated using an electronic spreadsheet (Rolim et al. 1998) following the procedure of Thornthwaite and Mather (1955) to determine the real evapotranspiration (ETr). The climatological water balance in the two soybean growing seasons is shown in Fig. 2.

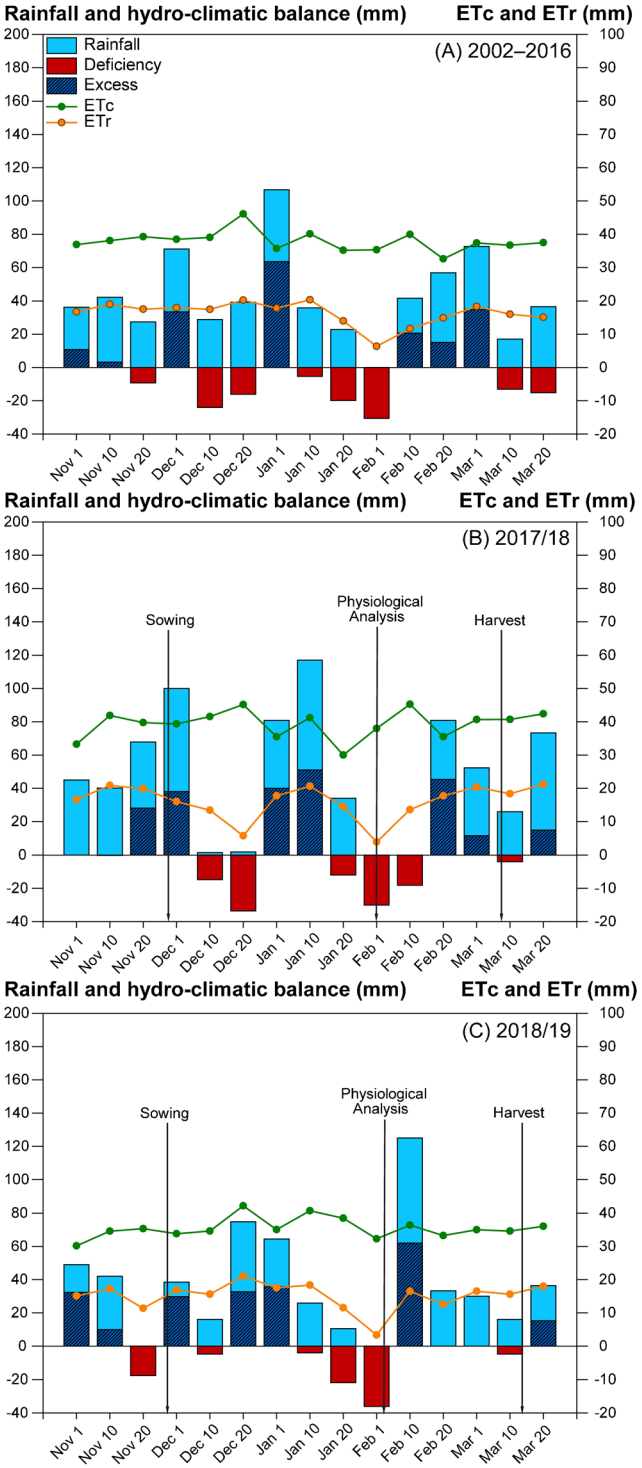


Fig. 2 Climatological water balance at Botucatu-SP, Brazil, (**A**) from 2002 to 2017 and during the soybean crop cycles, (**B**) in 2017/2018 and (**C**) in 2018/2019. ETc, crop evapotranspiration; ETr, real evapotranspiration.

2.4 Soil chemical properties analysis

In October 2018 (24 months after the fourth soil amendment application), eight individual soil samples were randomly collected from each plot at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m using a core sampler with an inner diameter of 50 mm. The samples from a single depth were combined into one sample per depth per plot. The samples were

dried, sieved (2 mm), and analyzed according to Cantarella et al. (1998). Soil pH was measured using a glass electrode at a soil:0.01 M $CaCl_2$ solution ratio of 1:2.5 (v/v) (Quaggio and van Raij 2001). SOM was determined according to the Walkley–Black method (Walkley and Black 1934). Total acidity (H + Al) was estimated using Shoemaker–McLean–Pratt (SMP) solution (Shoemaker et al. 1961). Exchangeable Al^{3+} was extracted using 1 M KCl solution and quantified by the titrimetric method with 0.025 M NaOH (Bertsch and Bloom 1996). Exchangeable Ca^{2+}, Mg^{2+} and K^+ were extracted with ion exchange resin (Quaggio and van Raij 2001) and determined by atomic absorption spectroscopy (AAS). Sulfate (SO_4^{2-}-sulfur) was extracted with 0.01 M calcium phosphate at a 1:2.5 (v/v) soil:solution ratio; the suspension was shaken for 30 min and filtered (Whatman no. 42) before determination of sulfate by a turbidimetric method (Bardsley and Lancaster 1960). Available phosphorus was extracted with ion exchange resin (van Raij et al. 2001) and quantified by a spectrophotometric method. Cationic micronutrients (Fe, Mn, Cu, and Zn) were extracted by a solution containing 0.005 M diethylenetriaminepentaacetic acid (DTPA; pH 7.3), 0.1 M triethanolamine (TEA), and 0.01 M $CaCl_2$ and determined by AAS (van Raij et al. 2001). The results were used to calculate the sum of exchangeable bases (SB) as the sum of Ca^{2+}, Mg^{2+}, and K^+. Cation exchange capacity was calculated as the sum of (H + Al) + SB. BS was calculated as the ratio of SB to CEC and expressed as a percentage. For the determination of SOM and P, Fe, Mn, Cu, and Zn concentrations, only the soil sampled at a depth of 0.0–0.2 m was analyzed.

2.5 Root dry matter

In both growing seasons, at soybean full flowering (R2 phenological stage) (Fehr et al. 1977), eight root subsamples were collected randomly from each plot and combined to form a composite sample. The samples were collected from plant rows and in the middle of the inter-rows of each subplot. A galvanized steel probe with an 82-mm-diameter cutting tip was used at depths of 0.0–0.1, 0.1–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0 m. The soybean roots were washed under a flow of swirling water over a 0.5-mm mesh sieve and separated from other plant materials after washing. The root samples were then dried in an oven at 60 °C for 72 h to determine root dry matter, which was expressed in g m^{-3} and subsequently estimated as Mg ha^{-1} in the 0.0–1.0-m layer. The root dry matter distribution was calculated by multiplying the ratio of root dry matter in each layer to total root dry matter by 100.

2.6 Nutrient concentrations in leaves and grains

Nutrient concentrations were analyzed in diagnostic leaves of soybean at full flowering (R2 soybean phenological stage) (Fehr et al. 1977) and grains after harvest. The diagnostic leaves of soybean were the third fully expanded leaf from the shoot apex. Leaves and grains were dried in an oven at 65 °C to constant weight and then ground in a Willey-type mill with a 1-mm screen for macro- and micronutrient analyses. N (leaves and grains) was extracted by sulfuric acid digestion, and the content was determined by the Kjeldahl method. Phosphorus, K, Ca, Mg, S, Fe, Mn, Cu, and Zn (only leaves) were extracted by nitroperchloric acid digestion and determined by AAS as described by AOAC (2016). Crude protein was determined by multiplying the grain N concentration by 6.25 (Krul 2019).

2.7 Gas exchange parameters

Gas exchange assessments were performed using diagnostic leaves of fifteen soybean plants per plot at full flowering (R_2 soybean phenological stage) (Fehr et al. 1977) and a Portable Infrared Gas Analyzer CIRAS-3 Portable Photosynthesis System (PP Systems Inc., Amesbury, MA, USA). The readings began after the air temperature in the chamber was adjusted to 28 °C with 380 ppm CO_2 and 1000 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) supplied by LED lamps. The minimum equilibration time before performing the reading was 3 minutes. The measurements were performed between 9:00 and 11:00 am. Then the net photosynthetic rate expressed as the area (*A*; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (*gs*; mol H_2O m^{-2} s^{-1}), leaf transpiration (*E*; mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration in the substomatal chamber (*ic*; mmol CO_2 mol^{-1} air), and water use efficiency (WUE; μmol CO_2 (mmol H_2O)$^{-1}$) were determined.

2.8 Physiological analysis of plant leaves

The leaves used for gas exchange analysis were subsequently used for physiological and biochemical analyses. The materials collected were placed in liquid nitrogen and stored at −80 °C until biochemical analysis.

2.8.1 Photosynthetic pigments

Photosynthetic pigments (chlorophyll *a*, *b*, total chlorophyll, and carotenoids) were determined using five discs cut between the edge and the central vein of the soybean leaves using a paper punch (0.5 cm in diameter). Following Lichtenthaler (1987), the discs were stored for 24 h in 2 mL of N,N-dimethylformamide (DMF) in capped glass vials wrapped in paper-lined aluminum foil, The concentrations of chlorophyll *a*, *b* and carotenoids were quantified using a spectrophotometric method at wavelengths of 664, 647, and 480 nm, respectively.

2.8.2 Rubisco (EC 4.1.1.39), and sucrose synthase (Susy, EC 2.4.1.13) activities

Based on the gas exchange data collected in the first growing season, we decided to evaluate the activities of the enzymes Rubisco and Susy in the second growing season to provide support for the previously obtained data.

Total Rubisco activity was measured according to the method described by Reid et al. (1997). Frozen plant material (0.3 g) was extracted with 1.5 mL of buffer containing 58 mM potassium phosphate and 1 mM ethylenediaminetetraacetic acid (EDTA). The material was centrifuged at 14,000 rotations per minute (rpm) for 25 min at 4 °C, and the supernatant was maintained at 4 °C (Reid et al. 1997). The incubation buffer for Rubisco consisted of 100 mM bicine-NaOH pH 8.0, 25 mM potassium bicarbonate ($KHCO_3$), 20 mM magnesium chloride, 3.5 mM ATP, 5 mM phosphocreatine, 0.25 mM NADH, 80 nkat glyceraldehyde-3-phosphate dehydrogenase, 80 nkat 3-phosphoglyceric phosphokinase, and 80 nkat creatine phosphokinase. A 70-μL aliquot of the supernatant was incubated with 900 μL of the incubation buffer for 5 min at 30 °C in the absence of ribulose-1,5-bisphosphate (RuBP) to enable carbamylation of Rubisco. NADP oxidation was initiated by adding 30 μL of 16.66 mM RuBP directly into the cuvette. Readings were carried out using a spectrophotometric method at a wavelength of 340 nm. Rubisco activity was calculated from the difference in the absorbance readings at 0 and 1 minute (without removing the cuvette from the spectrophotometer) is and expressed in μmol min^{-1} mg $protein^{-1}$.

Susy activity was determined by extracting 0.5 g of frozen plant material with extraction buffer containing 50 mM HEPES buffer pH 7.0, 2 mM $MgCl_2$, 2 mM dithiothreitol (DTT), and 1 mM EDTA according to Dejardin et al. (1997). After centrifugation at 14,000 rpm for 20 min at 4 °C, Susy buffer containing 0.1 M MES buffer pH 6.0, 5 mM $MgCl_2$, 0.3 M sucrose, and 5 mM uridine 5′-trihydrogen diphosphate (UDP) was added to 0.5 mL of the supernatant in extraction buffer and incubated at 37 °C for 30 min. The incubation was stopped by adding 100 μL of 30% potassium hydroxide (w/v) and heating at 100 °C for 5 min. Readings were carried out using a spectrophotometric method at a wavelength of 540 nm, and the results were expressed in μmol sucrose g^{-1} fresh weight (FW) h^{-1}.

2.8.3 Sucrose concentration

The sucrose concentration was determined from 1 g of frozen plant material extracted in 10 mL of MCW solution (60% methanol, 25% chloroform, and 15% water; (v/v/v)). The homogenized material was centrifuged at 8000 rpm for 10 min at 4 °C. An aliquot of 4 mL of the supernatant was removed and mixed with 1 mL chloroform + 1.5 mL distilled water. After the separation phase, the sucrose content was determined in the aqueous phase as described by Bieleski and Turner (1966). To quantify sucrose, 50 μL of extract, 500 mL of 30% KOH, and 2 mL of concentrated H_2SO_4 were added to a glass tube. The mixture was homogenized by vortexing and heated at 100 °C for 10 min. Readings were carried out using a spectrophotometric method at a wavelength of 490 nm. The sucrose concentration was determined by reference to a sucrose standard curve and expressed in mg g^{-1} FW.

2.8.4 Hydrogen peroxide

The concentration of hydrogen peroxide (H_2O_2) was determined by reaction with potassium iodide (KI) according to Alexieva et al. (2001). Frozen soybean leaves were ground in liquid nitrogen with a mortar and pestle, and 4 mL of 0.1% trichloroacetic acid (TCA) (w/v) were added. The homogenized material was centrifuged at 12,000 rpm for 15 min at 4 °C. For the reaction, 200 μL of supernatant, 200 μL of 100 mM potassium phosphate buffer pH 7.5, and 800 μL of KI solution (1 M) were combined and kept on ice for 1 h. After warming to room temperature, readings were carried out using a spectrophotometric method at a wavelength of 390 nm. The leaf concentration of H_2O_2 was calculated by reference to a standard curve and expressed in μmol g^{-1} FW.

2.8.5 Lipid peroxidation

Lipid peroxidation was evaluated based on the production of metabolites reactive to 2-thiobarbituric acid (TBA), mainly malondialdehyde (MDA), according to Heath and Packer (1968). The extraction was carried out with 0.8 g of frozen plant material in 4 mL of 0.1% trichloroacetic acid (TCA; w/v) + 20% polyvinylpolypyrrolidone (PVPP; w/v). The homogenized material was centrifuged at 10,000 rpm for 15 min at 4 °C, and 250 μL of the supernatant was added to 1 mL of 20% TCA + 0.5% TBA. The samples were heated at 95 °C for 30 min and immediately placed on ice for 10 min. The material was centrifuged again for 10 min at 10,000 rpm. Readings were carried out using a spectrophotometric method at wavelengths of 535 and 600 nm, and the results were expressed in nmol MDA g^{-1} FW.

2.8.6 Total soluble protein

Leaf samples were homogenized in 100 mM potassium phosphate buffer (pH 7.5) (1 g of tissue to 3 mL of buffer) containing 1 mM EDTA, 3 mM DTT, and 0.04 g of PVPP (Azevedo et al. 1998). The resulting homogenate was centrifuged at 10,000 rpm for 30 min at 4 °C, and the supernatant was stored at −80 °C before determination of antioxidant

activity. The total protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as a standard and measured by a spectrophotometric method at a wavelength of 595 nm. The results and protein extract were used to determine the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR).

2.8.7 Superoxide dismutase (EC:1.15.1.1)

Superoxide dismutase (SOD) activity was determined according to Giannopolitis and Ries (1977). The reaction was composed of 2 mL of 50 mM potassium phosphate buffer (pH 7.8), 250 μL of 13 mM methionine, 200 μL of 75 mM nitroblue tetrazolium (NBT), 200 μL of 0.1 mM EDTA, 250 μL of 2 μM riboflavin, and 50 μL of protein extract and was incubated in a reaction chamber under illumination by a 15 W fluorescent lightbulb at 25 °C. The tubes were vortexed and placed inside the chamber (total absence of light) for 15 min to allow the formation of the blue formazan compound via the NBT photoreaction. A control for each sample was performed with the same mixture, but these test tubes were covered with aluminum foil to prevent light exposure. After 15 min, the material was vortexed. Readings were carried out using a spectrophotometric method at a wavelength of 560 nm, and the results were expressed in U SOD mg^{-1} protein.

2.8.8 Catalase (1.11.1.6)

Catalase (CAT) activity was determined according to the method described by Azevedo et al. (1998). The reaction medium was composed of 1 mL of 30 mM H_2O_2 solution in 100 mM potassium phosphate buffer (pH 7.5). The reaction was started by the addition of 25 μL of protein extract. Enzymatic activity was determined by the decomposition of H_2O_2 during a 2-min interval using a spectrophotometer at a wavelength of 240 nm. The results were expressed in μmol min^{-1} mg^{-1} protein.

2.8.9 Ascorbate peroxidase (EC:1.11.1.11)

Ascorbate peroxidase (APX) activity was determined as described by Gratão et al. (2008). The reaction medium was composed of 100 μL of protein extract and 80 mM potassium phosphate buffer solution (pH 7.0) containing 5 mM ascorbate (AsA), 1 mM EDTA, and 1.35 mM H_2O_2. Readings were carried out immediately using a spectrophotometric method at a wavelength of 290 nm for 2 min. The results were expressed in μmol min^{-1} mg^{-1} protein.

2.8.10 Glutathione reductase (EC 1.6.4.2)

Glutathione reductase (GR) activity was determined as described by Gomes-Junior et al. (2006). The reaction buffer (1.7 mL) was composed of 1 mL of 100 mM potassium phosphate buffer (pH 7.5), 500 μL of 1 mM nitrobenzoic acid (DTNB), 100 μL of 1 mM oxidized glutathione (GSSG), and 100 μL of 0.1 mM NADPH. A 50-μL aliquot of the protein extract was added and vortexed. The solution was then transferred to a cuvette, and the absorbance at 412 nm was immediately recorded for 2 min. The results were expressed in μmol min^{-1} mg^{-1} protein.

2.9 Shoot dry matter and grain yield

At soybean flowering (R2 phenological stage), shoots were collected from a 0.25-m^2 area of each plot. The samples were dried in an oven at 60 °C for 72 h to determine shoot dry matter. At harvest, grain yield was evaluated based on the relationship between the mass of grains obtained in the interior (5.4 m^2) of each plot and their water content (130 g kg^{-1} of water).

2.10 Statistical analysis

All data were first checked for both normality and homoscedasticity. Then, all data were subjected to analysis of variance (F test), and the means of all treatments were compared by the t-test (Fisher's least significant difference (LSD) at $p \leq 0.05$). Results were expressed as the mean ± standard error of the mean. Comparisons of the means of soil chemical properties were conducted for each soil layer to assess treatment effects. Comparisons were not made between different soil depths. Redundancy analysis (RDA) was used to determine the correlations of carbon metabolism and oxidative stress with soil chemical properties. The Monte Carlo permutation test was applied with 999 random permutations to verify the significance of the effects of soil chemical properties on physiological responses. One-way PERMANOVA (Anderson 2005) was used to group the treatments by similarity. The correlation heatmap was constructed by calculating the Pearson's correlation coefficients ($p \leq 0.05$), and only significant correlations are shown.

3 Results

3.1 Climatic conditions

Pluvial precipitation from sowing to soybean physiological maturity was 468 and 418 mm in the first and second growing seasons, respectively (Fig. 2). However, the distribution of rain throughout the crop cycle was uneven, with two short dry spells with a negative water balance between November

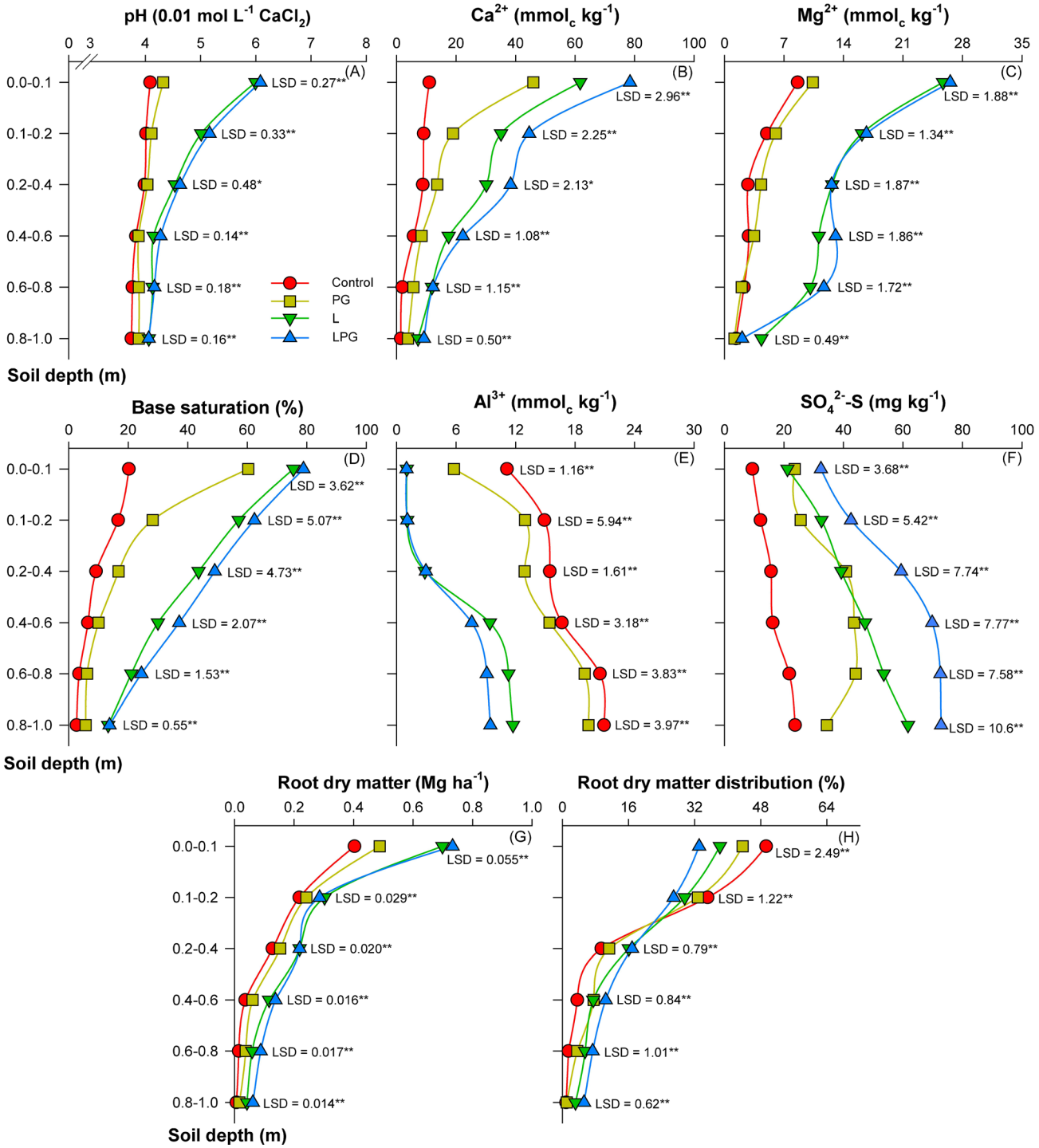


Fig. 3 Changes in soil chemical properties ((**A**) soil pH, (**B**) Ca^{2+}, (**C**) Mg^{2+}, (**D**) base saturation (BS), (**E**) Al^{3+} and (**F**) SO_4^{2-}-sulfur) and soybean root growth ((**G**) root dry matter and (**H**) root dry matter distribution) in the different treatments (control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)). Single and double asterisks indicate statistical significance at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to the LSD (least significant difference) test. Soil chemical properties and root growth were compared between treatments for each soil depth.

and February in each growing season. In both growing seasons, the first dry spell occurred after the emergence of seedlings, and the second dry spell spanned the beginning of flowering until pod formation (R1–R4 soybean phenological stage). Specifically, in the first growing season, the first dry spell occurred in the final two 10-day periods of December (accumulated rainfall = 3.4 mm; ETc = 43.4 mm; ETr = 19.2 mm; accumulated water deficit (AWD) = −48.3 mm), corresponding to the vegetative period. The second dry spell encompassed the final 10-day period of January and the first two 10-day periods of

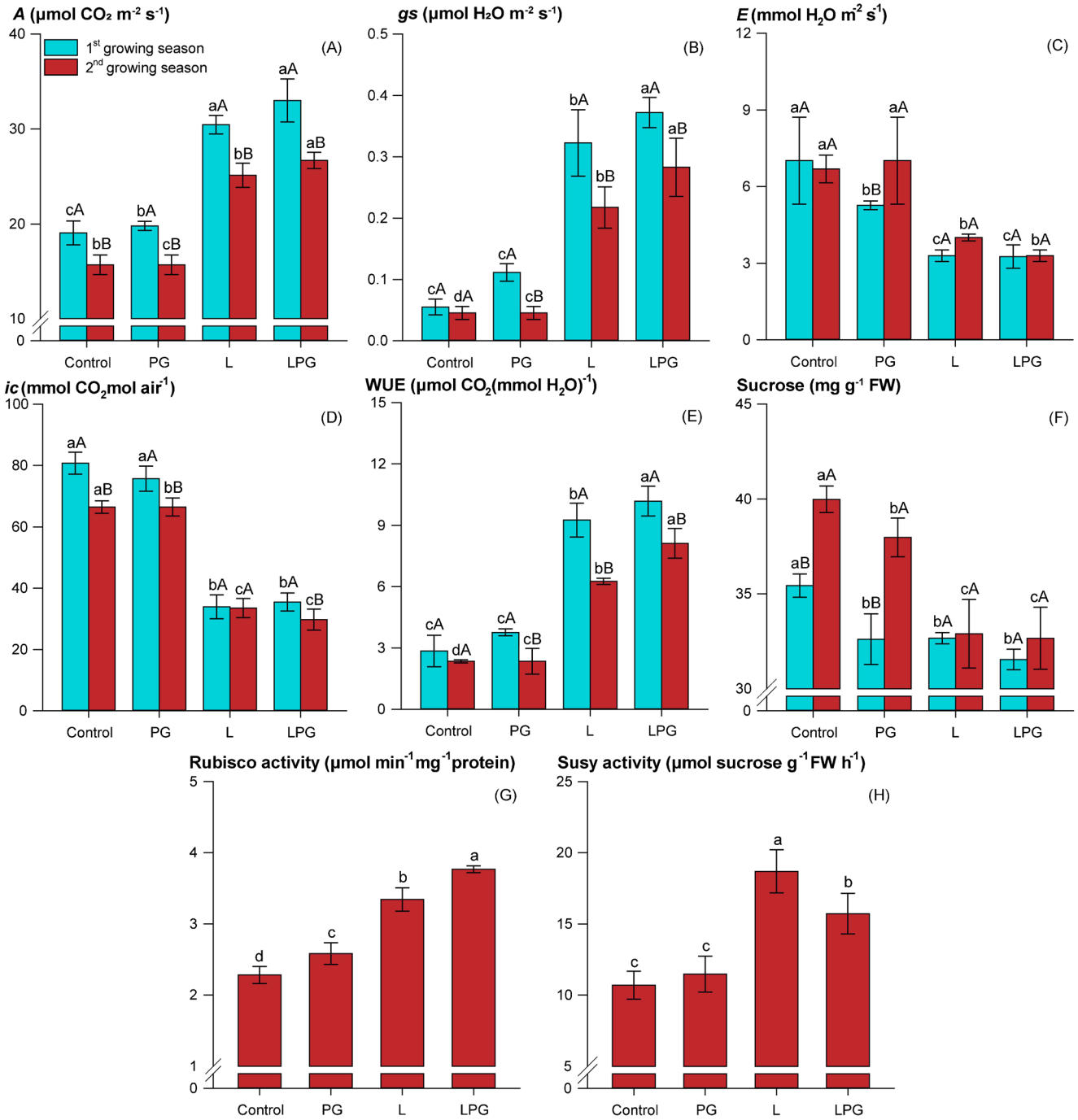


Fig. 4 Effects of the different treatments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] on (**A**) net photosynthetic rate (*A*), (**B**) stomatal conductance (*gs*), (**C**) transpiration rate (*E*), (**D**) internal CO_2 concentration (*ic*), (**E**) water use efficiency (WUE), (**F**) sucrose concentration, (**G**) Rubisco activity, and (**H**) Susy activity in soybean leaves. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's *t*-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

February (accumulated rainfall = 35 mm; ETc = 37.7 mm; ETr = 21.3 mm; AWD = −60.2 mm), corresponding to full flowering (R2) to pod formation (R4). In the second growing season, the first dry spell occurred in the first 10-day period of December (accumulated rainfall = 16.1 mm; ETc = 34.6 mm; ETr = 31.2 mm; AWD = −4.8 mm), corresponding to the vegetative period. The second dry spell included the final two 10-day periods of January and the first 10-day period of February (accumulated rainfall = 36.4 mm; ETc = 37.2 mm; ETr = 22.2 mm; AWD = −61.9 mm), corresponding to the beginning of flowering (R1) to pod formation (R4).

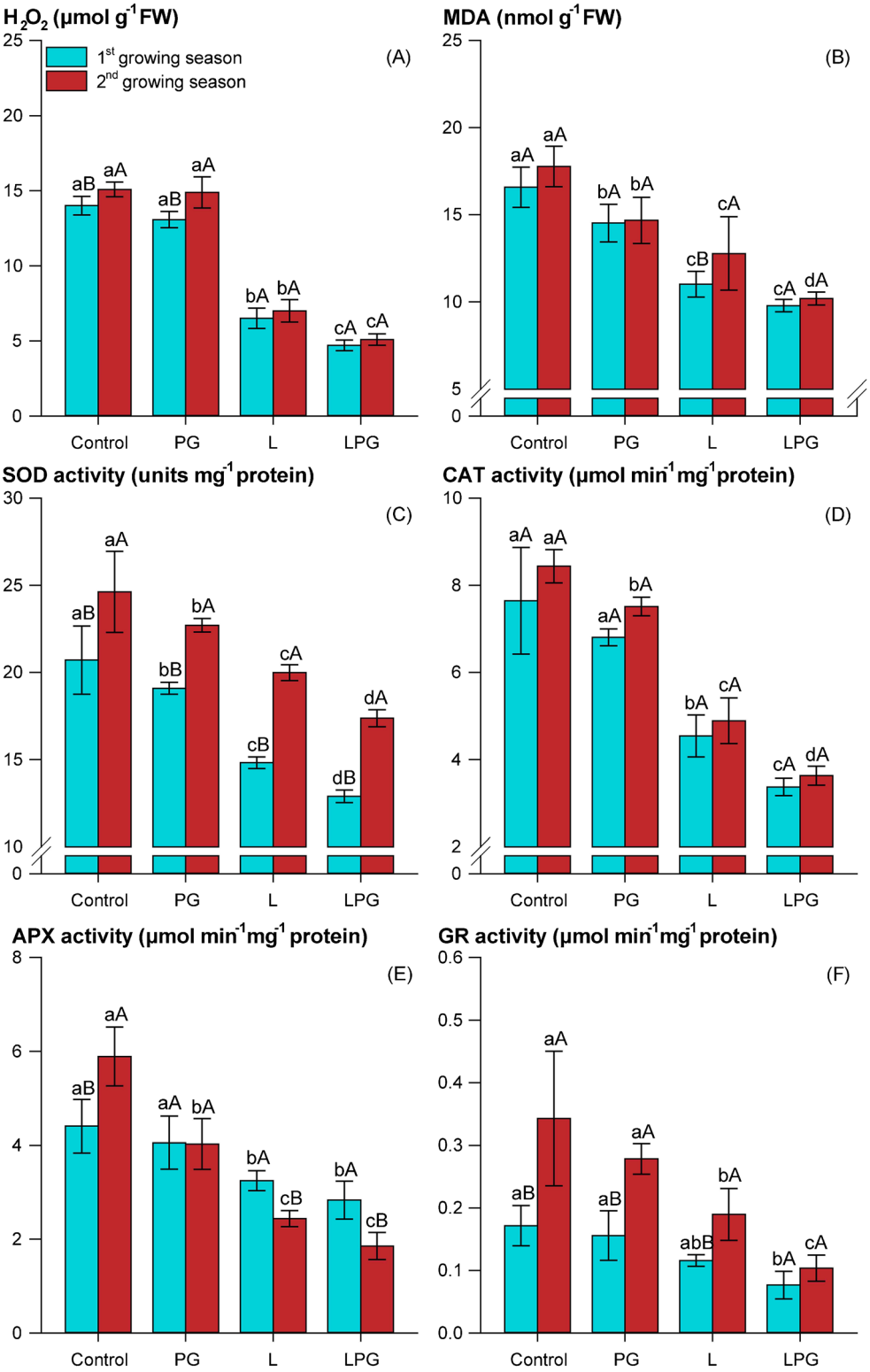


Fig. 5 Effects of the different treatments (control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)) on (**A**) hydrogen peroxide (H_2O_2), (**B**) malondialdehyde (MDA), (**C**) superoxide dismutase (SOD), (**D**) catalase (CAT), (**E**) ascorbate peroxidase (APX), and (**F**) glutathione reductase (GR) activity in soybean leaves. Different lowercase letters indicate significant differences between treatments, and different uppercase letters indicate significant differences between growing seasons by Student's *t*-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

3.2 Soil chemical properties and root development in the soil profile

At 24 months after the final application of L and PG in 2016, all soil fertility parameters differed significantly ($p < 0.01$; Table S2) among the treatments at all depths (Fig. 3). Surface application of L (regardless of PG addition) reduced soil acidity, even in deeper soil layers (Fig. 3A). As expected, application of PG alone did not change soil pH in any soil layer compared with the control.

All soil amendments increased the calcium concentration compared with the control (Fig. 3B), but the effects of L and LPG were superior to those of PG at all depths. Ca^{2+} levels were higher in LPG-amended soil than in L-amended soil up to a depth of 0.6 m and were similar in these two treatments in the 0.6–1.0-m layer. Amendment with L increased Mg^{2+} levels compared with the control regardless of the application of PG, but in the 0.4–0.6- and 0.8–1.0-m layers, LPG provided higher availability of Mg^{2+} compared with L (Fig. 3C).

Similar to the effects on Ca^{2+} and Mg^{2+}, L and LPG application increased BS levels at all soil depths compared with the PG and control treatments (Fig. 3D). BS values were slightly higher in the LPG treatment than in the L treatment at all depths except the deepest layer (0.8–1.0 m), whereas BS was similar in the two treatments. BS was higher in the PG treatment than in the control throughout the soil profile, with the exception of the 0.8–1.0-m layer.

Compared with the control, the concentration of exchangeable Al^{3+} was strongly reduced at all soil depths in the treatments that received L (L and LPG) and up to 0.4 m in the PG treatment (Fig. 3E). SO_4^{2-}-sulfur concentrations were highest at all soil depths in the LPG treatment, followed by the L and PG treatments, in which SO_4^{2-}-S concentrations were similar up to 0.6 m (Fig. 3F). At depths greater than 0.6 m, the SO_4^{2-}-S concentration was higher in the L treatment than in the PG treatment. SOM and P contents in the 0.0–0.2-m layer were significantly higher ($p < 0.01$; Table S3) in the L and LPG treatments than in the control and PG treatments. By contrast,

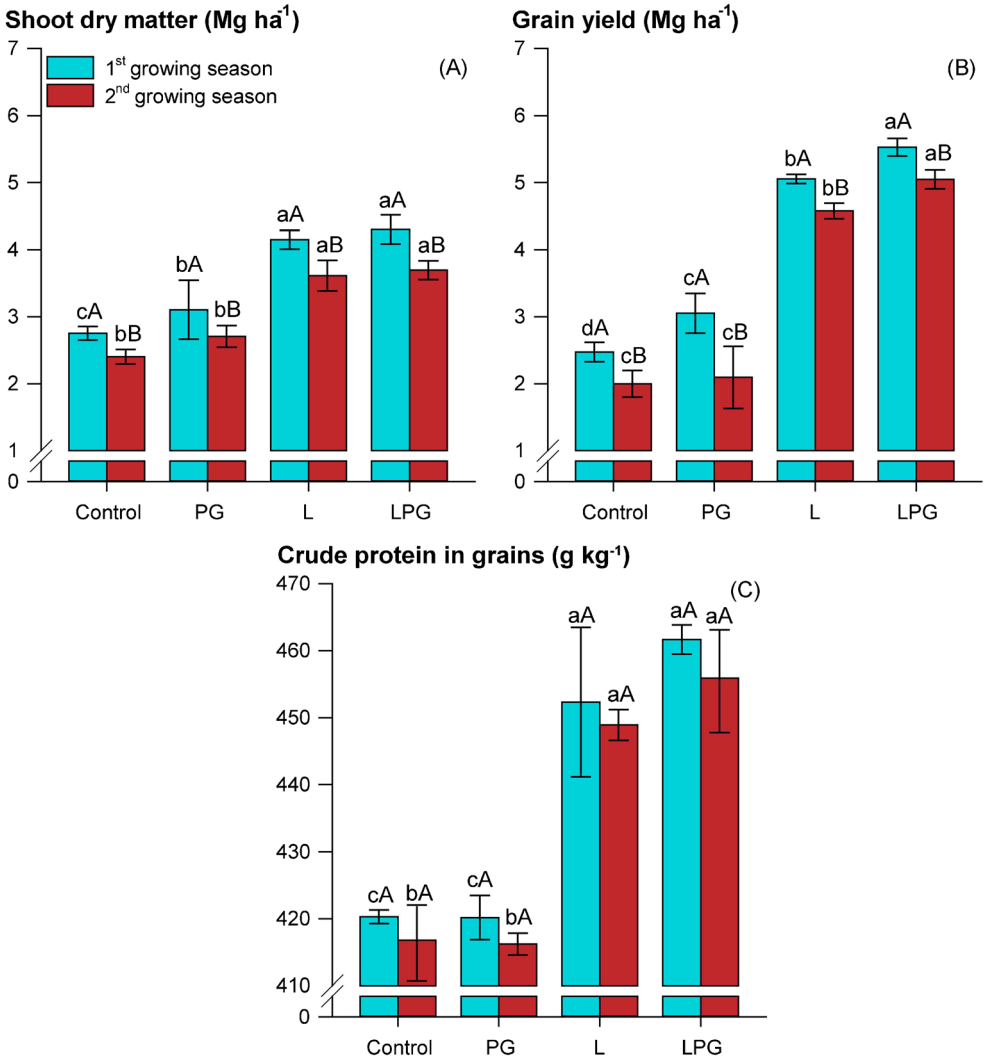


Fig. 6 Effects of the different treatments (control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)) on (**A**) shoot dry matter, (**B**) grain yield, and (**C**) crude protein in grains of soybean. Different lowercase letters indicate significant diff d different uppercase letters indicate significant differences between growing seasons by Student's *t*-test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

the concentrations of cationic micronutrients (Fe, Mn, Cu, and Zn) were significantly lower ($p < 0.01$; Table S3) in the L and LPG treatments.

There was no significant effect of the soil amendment × growing season interaction ($p > 0.05$; Table S4) on soybean root development parameters (Fig. 3 G and H). Application of L increased root dry matter production in all layers. In addition, synergetic effects of L and PG on root dry matter production were observed (Fig. 3G) in the uppermost layer (0.0–0.10 m) and at depths greater than 0.6 m. The proportion of roots distributed at a depth of 0.0–0.2 m was highest in the control treatment, followed by the PG treatment (Fig. 3H); consequently, the proportion of roots distributed at depths greater than 0.2 m was lower in these treatments than in the L and LPG treatments. In the L and LPG treatments, the root distribution was more uniform throughout the soil profile, with a higher proportion of roots in deeper layers (0.6–1.0 m depth).

3.3 Nutritional status of plants

There was no significant effect of the soil amendment × growing season interaction ($p > 0.05$; Table S6) on the concentration of any nutrient in soybean leaves (Table S5). Compared with the control, application of L (regardless of the application of PG) increased the concentrations of all leaf macronutrients except S, which was higher in the PG treatment. Calcium and Mg concentrations were higher in soybean plants grown in LPG-amended soil than in those grown in L-amended soil. In addition, compared with the LPG treatment, soybean plants in the PG treatment had similar Ca concentrations but lower Mg concentrations. L also increased the K concentration compared with the control, but this increase was slightly smaller when L was combined with PG (LPG). The leaf concentrations of Mn and Zn were lower in the L and LPG treatments than in the PG and control treatments.

3.4 Photosynthetic metabolism

The patterns of concentrations of chlorophylls (*a*, *b*, and total) and carotenoids were similar (Fig. S1). There was a significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) on the concentrations of all pigments, and the application of L (regardless of PG addition) resulted in the largest increases in pigment concentrations in soybean leaves compared with the control. The concentrations of leaf pigments did not differ between the PG and control treatments and were lower in the second growing season than in the first growing season in these two treatments.

Gas exchange parameters in soybean leaves were positively influenced by the soil amendment × growing season interaction ($p < 0.01$; Table S7) (Fig. 4). The application of L, particularly in combination with PG (LPG), impacted leaf gas exchange by increasing *A* (Fig. 4A), *gs* (Fig. 4B), and WUE (Fig. 4E) and reducing *E* (Fig. 4C) and *ic* (Fig. 4D) compared with the PG and control treatments.

A significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) was observed for leaf sucrose concentrations, which were lower in the L and LPG treatments (Fig. 4F). Additionally, leaf sucrose concentrations were higher in the second growing season than in the first season in the PG and control treatments. By contrast, Rubisco activity in soybean leaves was highest in the LPG treatment, followed in order by the L, PG, and control treatments (Fig. 4G; Table S7). On the other hand, Susy activity was highest in soybean in the L treatment, followed by the LPG, PG and control treatments (Fig. 4H; Table S7).

3.5 Oxidative stress and antioxidant metabolism

The concentrations of H_2O_2 and MDA were significantly influenced ($p < 0.01$; Table S7) by the soil amendments in both growing seasons (Fig. 5A,B). Application of L or LPG reduced oxidative stress (H_2O_2 and MDA production) in soybean plants compared with the PG and control treatments. In general, leaf antioxidant enzyme activities were highest in soybean cultivated in PG-amended or untreated soil (control) (Fig. 5C–F). As oxidative stress increased, the activities of SOD (Fig. 5C), CAT (Fig. 5D), APX (Fig. 5E), and GR (Fig. 5F) increased ($p < 0.01$; Table S7) proportionally. In addition, the activities of these enzymes were higher in the second growing season than the first growing season, particularly for SOD and GR, regardless of the treatment.

3.6 Soybean shoot dry matter production, grain yield and crude protein in grains

There was a significant effect of the soil amendment × growing season interaction ($p < 0.01$; Table S7) for soybean shoot dry matter production and grain yield. Shoot dry matter and grain yield were higher in the first growing season in all treatments (Fig. 6A). Considering both growing seasons, on average, the treatments that received L (L and LPG) produced ~44% more shoot dry matter than the control and PG treatments. Compared with the control, the PG, L, and LPG treatments increased average grain yield over the two growing seasons by ~15, 116, and 140%, respectively (Fig. 6B). The crude protein concentration in grain did not differ between the growing seasons (Fig. 6) and was ~9.5% higher in the treatments that received L (L and LPG) than in the control and PG treatments (Fig. 6C).

3.7 Redundancy analysis and Pearson's correlation analysis of environmental factors (soil chemical properties) and soybean plant parameters

Redundancy analysis (RDA) was performed to identify the main soil factors involved in the responses of carbon fixation metabolism and antioxidant enzymes in soybean plants to the different soil amendment treatments (Fig. 7A). Soil fertility was responsible for 94% of all enzymatic variation. PERMANOVA analysis separated the treatments into three distinct groups ($p < 0.001$), with the control and PG treatments as group 1, the L treatment as group 2, and the LPG treatment as group 3. Monte Carlo permutation analysis indicated that pH ($F = 79.3$; $p < 0.001$), Ca^{2+} ($F = 7.65$; $p = 0.032$), and Mg^{2+} ($F = 3.93$; $p = 0.007$) were the soil factors that most positively influenced enzymes related to carbon metabolism, whereas Al^{3+} ($F = 2.85$; $p = 0.023$) was the soil factor responsible for increasing oxidative stress and antioxidant metabolism in soybean plants.

Correlation analysis among soil and plant parameters demonstrated that improving soil chemical properties (i.e., increasing pH, SOM, Ca^{2+}, and Mg^{2+} levels and reducing Al^{3+} levels) improved root development, carbon fixation and its covariates (i.e., pigments, gas exchange, Rubisco and Susy activity), reduced oxidative stress, and increased soybean yield (Fig. 7B).

4 Discussion

4.1 Weather conditions

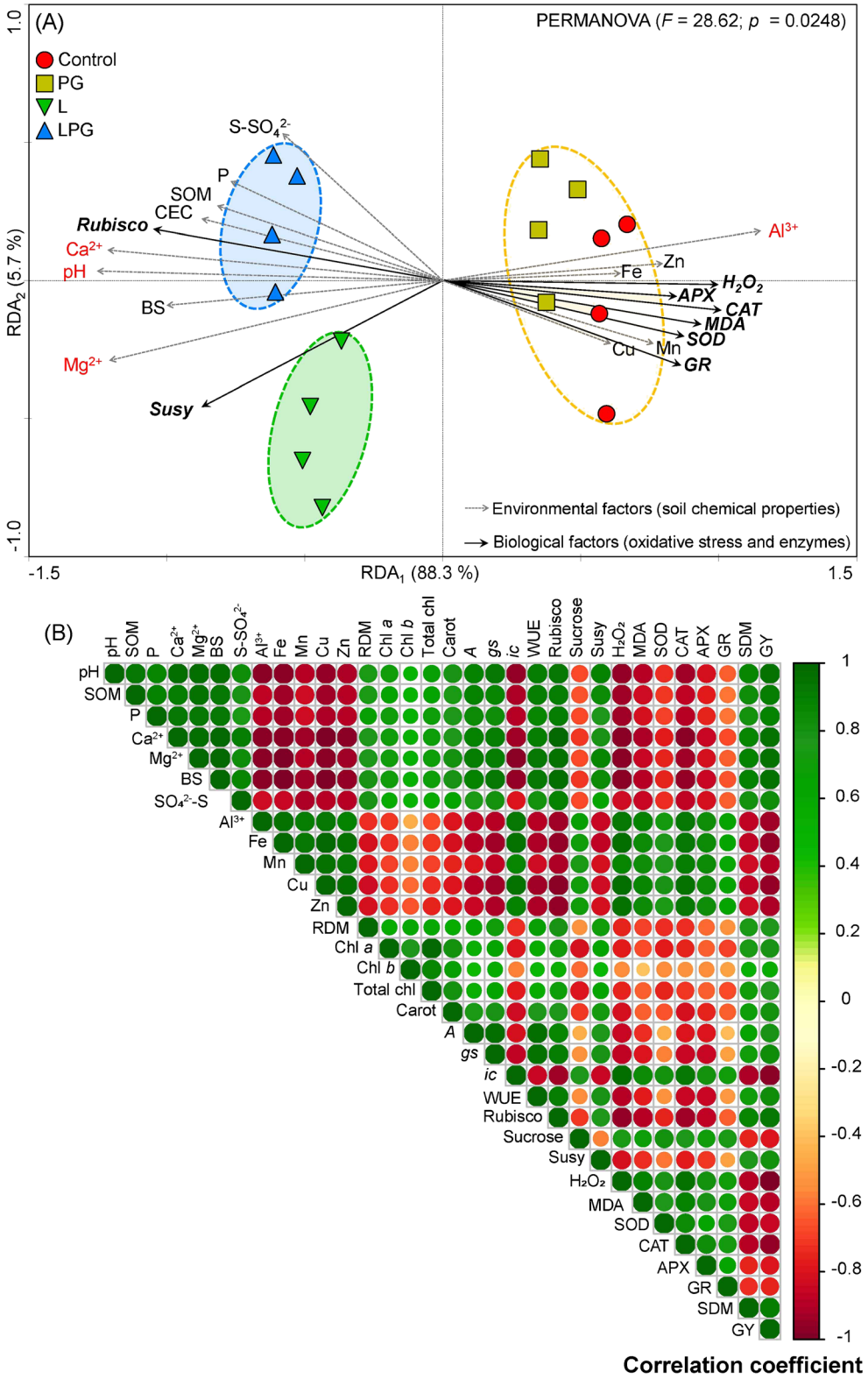
In both growing seasons, rainfall was within the range considered appropriate (450 to 800 mm) to guarantee soybean yield potential according to the recommendations of Farias et al. (2007). However, the water balance was negative in December and January in both seasons, which corresponds to the flowering and initial pod development stages. Short dry periods during the summer cropping season are common in tropical regions (Cunningham 2020). When temperatures and evapotranspiration are high, even short dry periods can cause considerable crop yield losses, especially when such dry periods coincide with critical periods of crop development, such as germination, flowering, and grain filling (Basu et al. 2016). The higher Al^{3+} content in low-fertility acidic tropical soils affects root growth (Bossolani et al. 2021). Soil amendments can significantly enhance drought tolerance by not only correcting soil acidity and increasing root growth but also improving nutrient availability (Carmeis Filho et al. 2017).

4.2 Soil chemical properties, root development, and nutritional status of plants

Over the 16-year experimental period, surface-applied L proved to be a viable practice for improving soil chemical properties and alleviating acidity in the soil profile under NTS. Numerous studies have demonstrated that, over the long term, L amendment efficiently increases root growth, improves soil aggregation (Carmeis Filho et al. 2018; Costa et al. 2021; Ferrari Neto et al. 2021), reduces runoff and erosion (Tebebu et al. 2020), and increases soil fertility in the uppermost soil layers (Nora and Amado 2013; Grover et al. 2017; Auler et al. 2019). However, the effects of L on soil chemical properties in subsoil layers have not been comprehensively examined. Overall in the present study, the effects of PG application alone on soil attributes compared with the control were small (with the exception of soil pH, which was unchanged) and were most pronounced in the superficial layers. Although PG alone was not efficient in reducing soil acidity, combining this soil amendment with L (LPG) potentiated the effects of L (e.g., increased Ca^{2+}, Mg^{2+}, BS, and SO_4^{2-}-S levels and decreased Al^{3+} levels), suggesting that PG is an important complement to L in tropical soils (Crusciol et al. 2019; Bossolani et al. 2020, 2021; Moretti et al. 2020). PG is a widely available and inexpensive soil amendment (Zoca and Penn 2017) that provides numerous benefits to agricultural systems, especially when combined with L (Crusciol et al. 2019; Bossolani et al. 2020, 2021). Upon application to soil, PG quickly dissociates into SO_4^{2-}-S, which reduces the harmful effects of Al^{3+} throughout the soil profile (Reis et al. 2018), and Ca^{2+} (fertilizing action).

The alleviation of soil acidity by L correlated significantly with increased nutrient availability. In the uppermost layers (0.0–0.2 m), application of L (regardless of PG addition) increased P availability and SOM input despite reducing Fe, Mn, Cu, and Zn levels. SOM is the main source of micronutrients for plants, especially in the arable layer (0.0–0.2 m), where SOM content is higher (Fageria and Stone 2008). SOM is also the main source of SO_4^{2-}-S in soils, and the increase in the S concentration in L-amended soil can be explained by the increase in SOM content in the uppermost soil layers due to the higher input of crop residues (shoot and root) over time (Kopittke et al. 2016). The decrease in micronutrient availability due to surface liming can be circumvented by regularly applying micronutrients (as done in this study) or applying conventional fertilizers ($N–P_2O_5–K_2O$) plus micronutrients in the same input.

The improvements in soil chemical quality were reflected in enhanced soybean root growth and well-nourished soybean plants. The increase in soybean root development is likely attributable to increased availability of exchangeable cations, mainly Ca^{2+}, in deeper layers and reduced Al^{3+} content (Ritchey et al. 1982; Costa et al. 2018). The proportion of roots distributed in



deeper layers was highest in the LPG treatment, followed by the L treatment, echoing the pattern of soil fertility parameters. Calcium is required during cell division and elongation (Ritchey et al. 1995), which are disrupted by Al^{3+} (Reis et al. 2018). Therefore, improving chemical attributes throughout the entire soil profile can significantly improve root development and distribution by reducing the concentration of roots in surface layers and encouraging root development in deeper layers of the

◀ **Fig. 7** (**A**) Redundancy analysis (RDA) of the correlations of enzymes related to carbon and antioxidant metabolism with soil chemical properties. The arrows indicate correlations between factors. The canonical axes are labeled with the percentage of total variance explained (%). The significance of correlations was evaluated by a Monte Carlo permutation test, and the significant soil properties are indicated by red color ($p \leq 0.05$). The colored dashed lines indicate significant clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). (**B**) Heatmap of correlations (Pearson) of soybean physiological, biochemical and agronomic parameters with soil chemical properties. Only significant correlations at $p \leq 0.05$ are shown. Soil organic matter (SOM), base saturation (BS), root dry matter (RDM), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total chl), carotenoids (Carot), net photosynthetic rate (*A*), stomatal conductance (*gs*), internal CO_2 concentration (*ic*), water use efficiency (WUE), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), shoot dry matter (SDM), and grain yield (GY).

soil. Longer and deeper roots can efficiently take up water and nutrients from deep layers (Rellán-Álvarez et al. 2016), as evidenced in this study by the significantly higher leaf concentrations of N, Ca, and Mg in soybean plants in the L and LPG treatments. Improving root growth and distribution is fundamental for reducing the negative effects of low water availability during crop development. Low water availability is common in tropical regions (Carmeis Filho et al. 2017) and was observed during both growing seasons in this study. In a region with constant rainfall and lower evapotranspiration rates (subtropical region), Caires et al. (2008) observed an increase in the proportion of roots at depths shallower than 0.20 m only. By contrast, root development increased at depths of up to 1.0 m under tropical conditions in the present study, highlighting the importance of amendment with L and PG to guarantee food production under unpredictable weather (Rellán-Álvarez et al. 2016) in a scenario of climate change.

4.3 Leaf pigments, gas exchange and carbon metabolism

Leaf pigment concentrations (Chl *a*, *b*, total and carotenoids) were highest in the treatments that received L (L and LPG), probably due to higher soil nutrient availability and nutrient uptake by soybean roots. In photosynthesis, chlorophylls are responsible for capturing light, which excites electrons used to produce reducing and energetic compounds (NADPH and ATP) for reactions of the Calvin cycle (Croft et al. 2017). Carotenoids act as antenna pigments for capturing light and protecting the photosynthetic apparatus against photodestruction by reactive oxygen species (ROS) during abiotic environmental stresses (Gómez et al. 2019).

Pearson correlation analysis indicated a positive relationship between leaf pigment concentrations and the photosynthetic potential of soybean plants. In the L and LPG treatments, the *A* rate increased to the detriment of soybean leaf pigment concentrations. Heat and water stress are exacerbated in tropical environments, resulting in greater declines in crop yields (Paustian et al. 2004). However, under the conditions of the present study, *gs* and WUE increased proportionally with the *A* rate. The *A* rate is mainly reduced through stomatal closure or metabolic impairment (Basu et al. 2016), as observed in the control and PG treatments. In these treatments, *gs* was significantly lower, whereas *ic* (indicating low CO_2 fixation) and *E* were high, suggesting dysfunction of stomatal control. Stomatal control is the immediate plant response to drought stress (Gupta et al. 2020).

In photosynthesis, reducing power and energy produced in the photochemical phase are used to fix CO_2, and this process is highly dependent on tight coordination of enzymatic activity and production of metabolic intermediates (Choudhury and Behera 2001). Metabolic impairment during low water and nutrient availability is mainly caused by changes in carbon metabolism (Basu et al. 2016). CO_2 fixation efficiency is highly dependent on Rubisco activity, which was higher in the L and, in particular, LPG treatments, corroborating the positive correlations of pigment concentrations with gas exchange. Our findings also indicated changes in source–sink relationships, which determine the grain yield of crops (Ceylan et al. 2016). Low translocation of carbohydrates from source to sink implies less filling of grains or even less capacity to promote root growth, reducing crop establishment under low water availability (Li et al. 2015). Thus, the sink strength (ability of an organ to import photoassimilates) (Basu et al. 2016) is much lower in plants established in low-fertility environments. Furthermore, the low Susy activity in soybean leaves in the control and PG treatments suggests that the process of cleavage of sucrose molecules by Susy (and invertases) for metabolism and conversion into energy and carbon skeletons for plant metabolism was greatly compromised by the poor soil fertility and nutrition (Stein and Granot 2019).

The main soil factors driving soybean carbon metabolism were verified by RDA and confirmed by correlation analysis. PERMANOVA segregated the treatments into three groups: LPG; L; and control and PG. This result indicates that PG application alone was not sufficient to improve soil attributes and, in turn, alter carbon metabolism in soybean plants compared with the control treatment. By contrast, L increased carbon metabolism, and the effects of L were even greater when combined with PG (LPG). Alleviation of soil acidity was the primary factor responsible for increasing Ca^{2+} and Mg^{2+} availability and reducing Al^{3+}, which enhanced Rubisco and Susy activities. Calcium and Mg are essential nutrients for photosynthesis and are involved in many other plant development pathways, such as cell division and elongation and resistance to environmental stress (Wang et al. 2019). Calcium participates in stomatal movements and regulates photosynthetic enzymes during carbon assimilation and chloroplast movement in photosynthetic cells depending on light conditions. Low Mg^{2+} availability decreases photosynthetic

capacity by reducing the activities of Rubisco and ATPases (Cakmak and Kirkby 2008), and Mg^{2+} is also an essential component of the chlorophyll molecule and plays a central role in partitioning of photoassimilates by plant tissues (Ceylan et al. 2016). Soybean plants are highly sensitive to Al^{3+} (Reis et al. 2018), as confirmed by the negative impacts of the high concentrations of Al^{3+} in the control and PG treatments on soybean plant parameters. At toxic concentrations, exchangeable Al^{3+} decreases plant root growth and WUE (Carmeis Filho et al. 2017; Reis et al. 2018), compromising crop development and yield.

4.4 Antioxidant metabolism

Continuous photosynthetic reactions under low water and nutrient availability result in the accumulation of ROS (Cuypers et al. 2010) such as singlet oxygen ($^1O_2^-$), hydroxyl radicals (OH^-), and H_2O_2 (Farmer and Mueller 2013). ROS can cause serious damage to the photosynthetic apparatus, resulting in lipid peroxidation (Loix et al. 2018). To mitigate the effects of oxidative stress, antioxidant enzymes are activated that catalyze reactions of ROS to reduce cell damage (Farooq et al. 2019). In the L and LPG treatments, the activities of the enzymes SOD, CAT, APX, and GR in soybean plants were low, indicating sufficient mitigation of ROS. On the contrary, the activities of these enzymes and H_2O_2 concentrations were high in the control and PG treatments, which demonstrated that the ability of these enzymes to eliminate ROS was inadequate (Farmer and Mueller 2013). In addition, excessive accumulation of carbohydrates in source tissues increases ROS production, especially in chloroplasts, limiting photosynthesis via a negative feedback effect (Cakmak and Kirkby 2008).

RDA and correlation analysis clearly indicated that soils with higher Ca^{2+} and Mg^{2+} availability had improved carbon metabolism and lower oxidative stress due to the roles of these nutrients in photosynthesis. In addition, the resistance of plants to environmental abiotic stress was higher in soils with higher Ca^{2+} and Mg^{2+} availability but reduced in soils with lower availability of these nutrients and higher Al^{3+} levels.

4.5 Soybean shoot dry matter production, grain yield and crude protein in grains

The differences in soil fertility, root development, and crop physiological responses among the treatments were reflected in soybean shoot dry matter and grain yield. The results demonstrated that combining L with PG (LPG treatment) is a viable strategy to improve the productive capacity of acidic tropical soils under NTS (Nora et al. 2017; Crusciol et al. 2019; Bossolani et al. 2020, b). Notably, the effects of the treatments on grain yield were greater than those on shoot dry matter, although the trends were similar. This difference can be explained by the compromised transport of carbohydrates over a long distance (source–sink) (Ceylan et al. 2016), which reduces grain filling. Grain filling is dependent on the translocation of carbohydrates from source-to-sink organs, a process mediated by Mg^{2+} nutrition (Cakmak and Kirkby 2008). In addition, sucrose is the main carbohydrate transported (via phloem) to grains, and the production of sucrose is strongly influenced by Mg^{2+} nutrition (Ceylan et al. 2016). Our results are consistent with these characteristics of grain filling, as the plants established in soils that received liming (L and LPG) had higher Mg^{2+} nutrition (higher Mg^{2+} availability and uptake by increasing root growth) and less sucrose accumulation in leaves (greater translocation efficiency), culminating in higher grain yield. In addition, the improvements in soybean metabolism and photoassimilate translocation to grains resulted in a significant increase in the concentration of crude protein in soybean grains. Crude protein concentration is an important characteristic related to grain quality and plays a critical role in human and animal nutrition (Karr-Lilienthal et al. 2004).

This study in a tropical region clearly demonstrated that liming benefits soil properties and soybean development. In addition, the combined application of L and PG (LPG) potentiated the beneficial effects of L, providing greater support for grain production, especially in periods of low water availability during soybean development.

5 Conclusions

This study makes an important contribution to understanding the long-term changes below- and aboveground in response to lime and phosphogypsum application. Our results demonstrate that liming is an important tool for improving acidic, low-fertility soils and that the effects of liming are enhanced by phosphogypsum amendment. The combined application of liming and phosphogypsum increased soybean root growth and the ability of plants to acquire water and nutrients from deeper soil layers. These findings have important implications for corrective soil management practices, particularly for the surface application of lime and/or phosphogypsum to increase soybean metabolism. The chemical properties of soil clearly impact soybean metabolism and production, and high-fertility soils enable high photosynthetic performance coupled with increased antioxidant activity. Long-term management with lime and phosphogypsum is therefore an effective approach to increase the grain yield of soybean cultivated in tropical regions predisposed to short dry spells.

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Authors' contributions J.W.B. and C.A.C.C. contributed to the design and execution of this research, data analysis, and writing and formatting the manuscript. L.G.M., A.G., J.R.P., L.B., R.G.V., J.C.C., E.F.C., T.J.C.A., and A.R.R. rewrote, discussed, and commented on the manuscript. All authors contributed significantly to this manuscript and approved it for publication.

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Data availability The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors whose names appear on this submission approved the version to be published and agree to be accountable for all aspects of the work and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interests The authors declare no competing interests.

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