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*Functional Plant Biology*

**Supplementary Material**

# Nitrate supply decreases fermentation and alleviates oxidative and ionic stress in nitrogen-fixing soybean exposed to saline waterlogging

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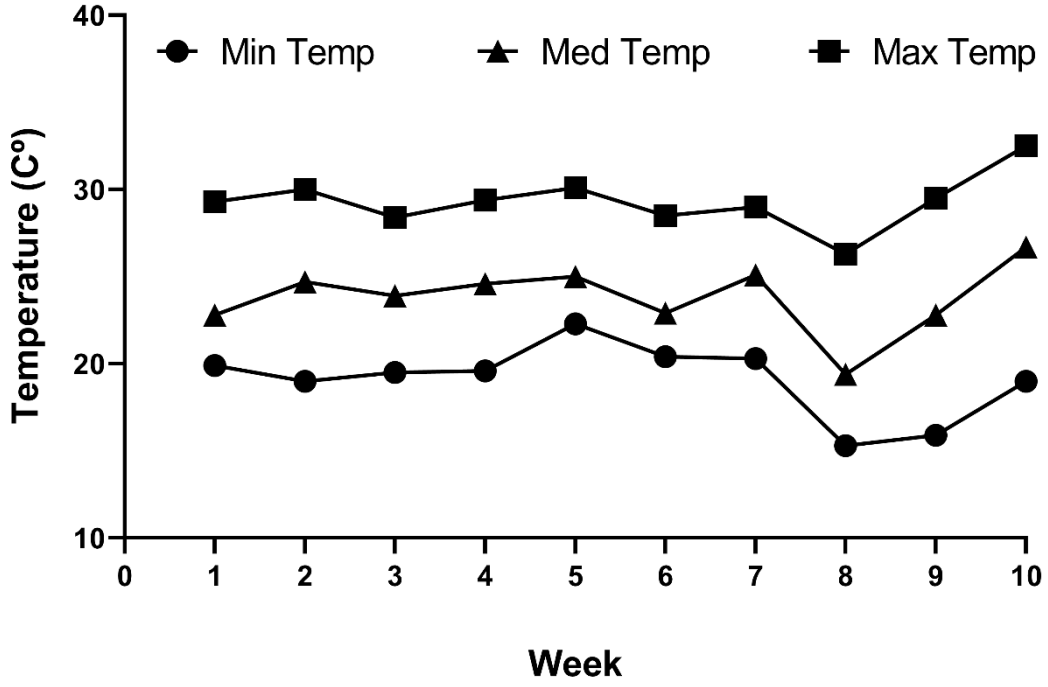


**Fig. S1.** Minimum daily temperature (Min Temp), medium daily temperature (Med Temp), and maximum daily temperature (Max Temp) during the entire experiment (January to February/2020). The plants were exposed to waterlogging and drainage between weeks nine and ten. Source: Agroclimatological Station of Pelotas, Empresa Brasileira de Pesquisa Agropecuária, Embrapa Clima Temperado, Pelotas, Brazil. Available at: http://agromet.cpact.embrapa.br/.

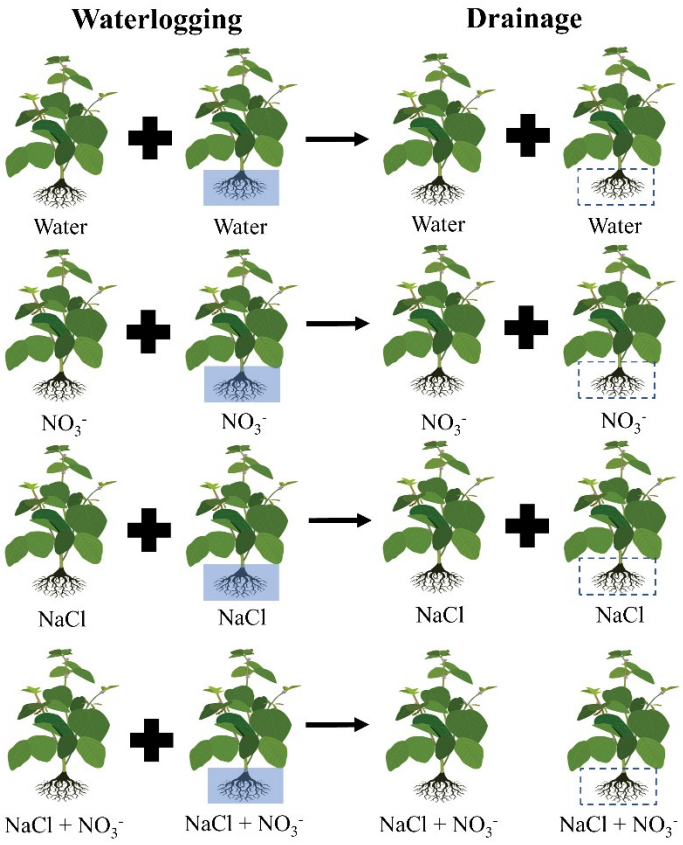


**Fig. S2.** Nodulated soybean plants at the R2 stage grown in the absence of mineral nitrogen exposed to waterlogging and different treatments: Water, $NO_3^-$ (3.4 mM), NaCl (50 mM), and NaCl + $NO_3^-$. The evaluations were carried out during waterlogging (six days) and drainage (two days). n=4.

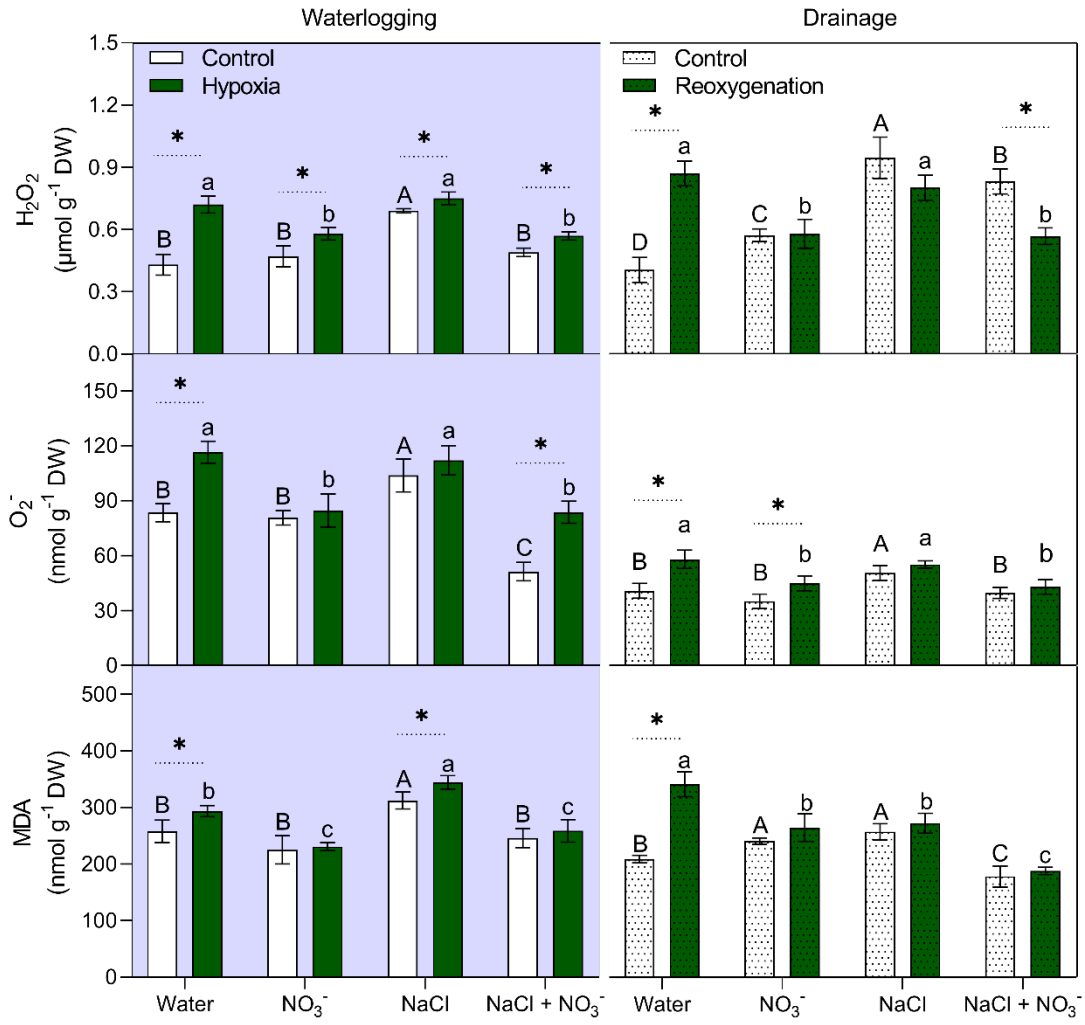


**Fig. S3.** Effect of nitrate ($NO_3$; 3.4 mM) supplementation on the levels of $H_2O_2$, $O_2^-$ and malondialdehyde (MDA) in leaves of soybean plants exposed to salinity (NaCl; 50 mM) during waterlogging (six days) or reoxygenation (two days). Values are mean ± SD, n = 4. Asterisks indicate significant differences between plants under hypoxia/reoxygenation and control conditions (*t*-test; $P<0.05$), distinct uppercase letters indicate significant differences among control plants, and distinct lowercase letters indicate significant differences among plants under hypoxia/reoxygenation (Scott-Knott; $P < 0.05$). DW, dry weight.

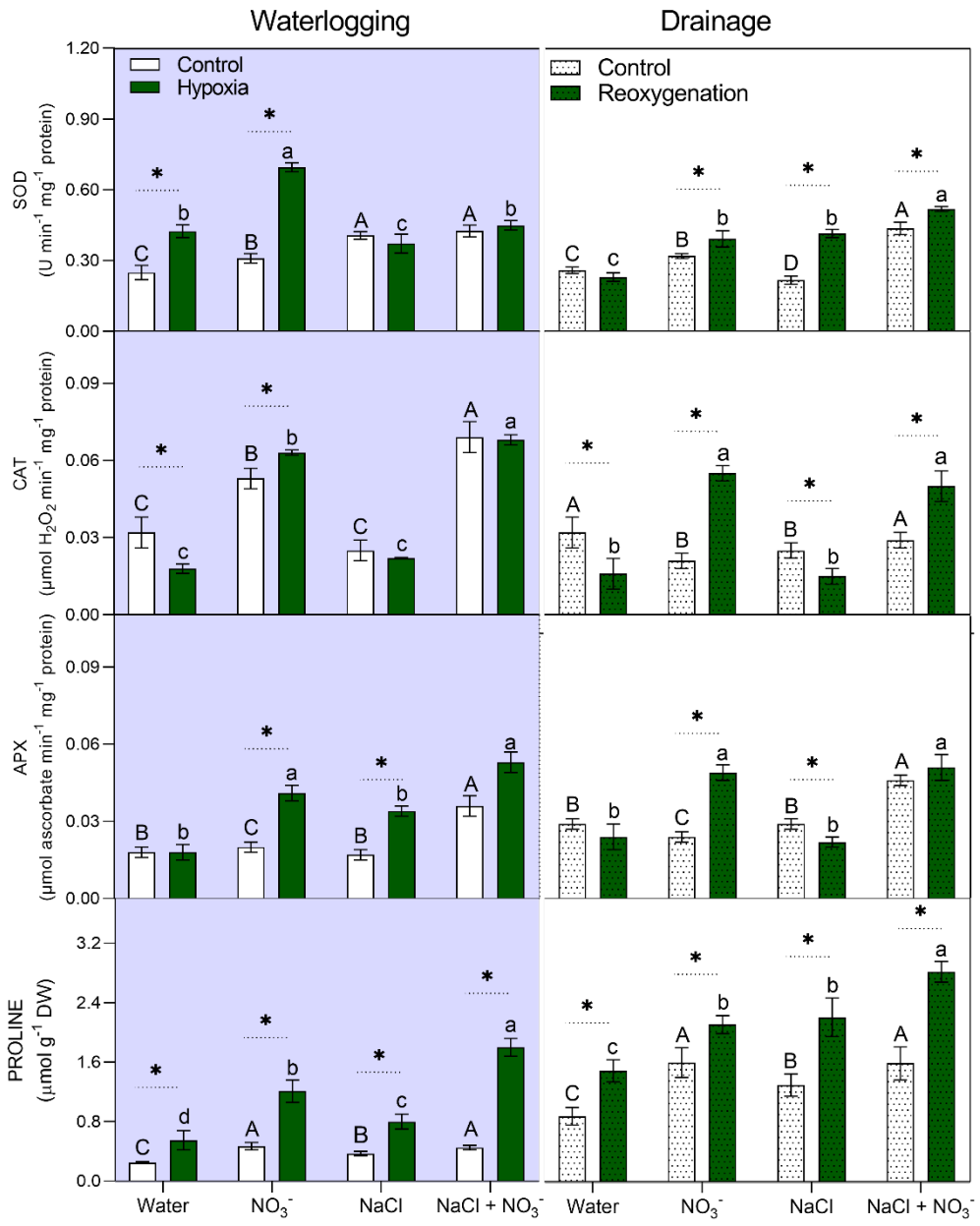


**Fig. S4.** Effect of nitrate ($NO_3$; 3.4 mM) supplementation in the activity of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) and levels of proline (PROLINE) in leaves of soybean plants exposed to salinity (NaCl; 50 mM) during waterlogging (six days) or drainage (two days). Values are mean ± SD, n = 4. Asterisks indicate significant differences between plants under hypoxia/reoxygenation and control conditions (*t*-test; $P<0.05$), distinct uppercase letters indicate significant differences among control plants, and distinct lowercase letters indicate significant differences among plants under hypoxia/reoxygenation (Scott-Knott; $P < 0.05$).

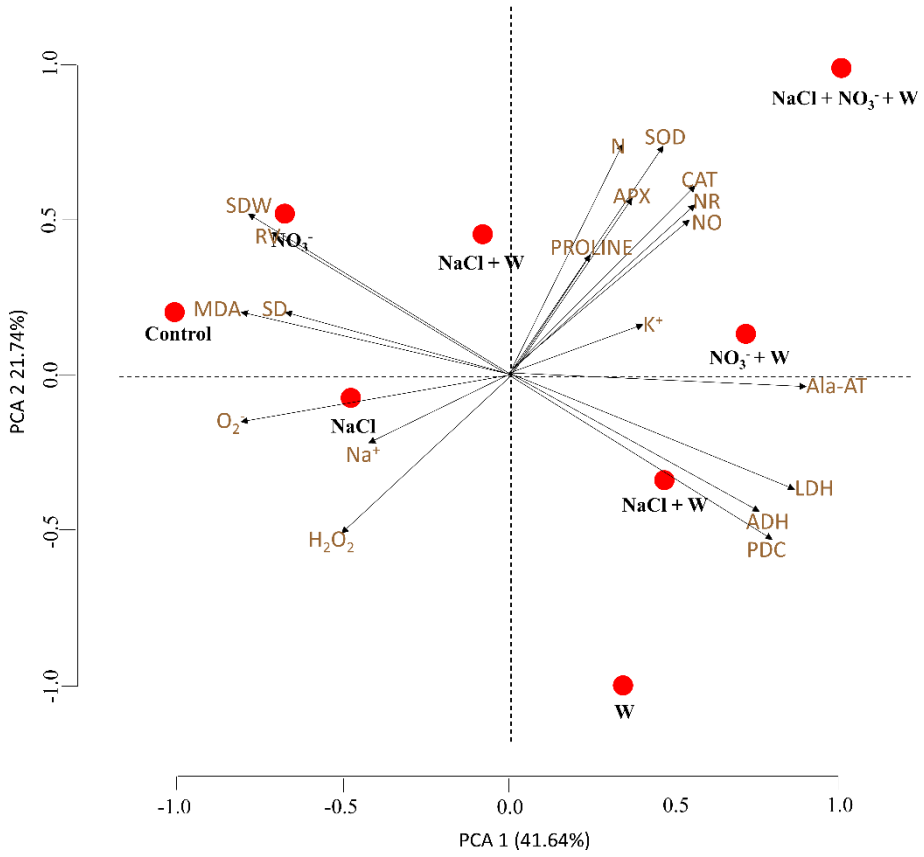


Fig. S5. Principal component analysis based on biochemical data of roots of soybean plants treated with nitrate ($NO_3^-$) and NaCl during six days of waterlogging (W). Control plants were not exposed to waterlogging neither NaCl nor $NO_3^-$ treatments. The data used in the analysis were the activity of nitrate reductase (NR), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH), alanine aminotransferase (Ala-AT), and pyruvate decarboxylase (PDC), the levels of $NO_3^-$, nitric oxide (NO), lipid peroxidation (MDA), superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), proline (PROLINE), sodium ($Na^+$), and potassium ($K^+$), and root volume (RV), root dry weight (RDW) and stem diameter (SD).

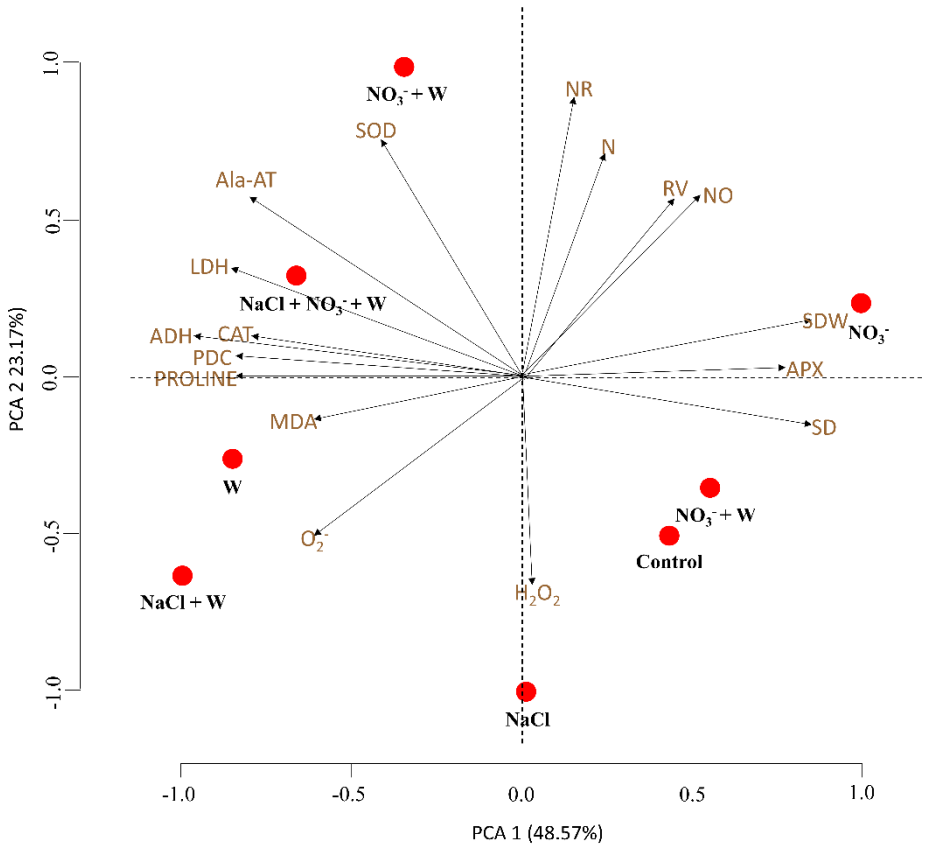


Fig. S6. Principal component analysis based on biochemical data of roots of soybean plants treated with nitrate ($NO_3^-$) and NaCl during reoxygenation (two days) after six days of waterlogging (W). Control plants were not exposed to waterlogging neither NaCl nor $NO_3^-$ treatments. The data used in the analysis were the activity of nitrate reductase (NR), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), lactate dehydrogenase (LDH), alcohol dehydrogenase (ADH), alanine aminotransferase (Ala-AT), and pyruvate decarboxylase (PDC), the levels of $NO_3^-$, nitric oxide (NO), lipid peroxidation (MDA), superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), proline (PROLINE), sodium ($Na^+$), and potassium ($K^+$), and root volume (RV), root dry weight (RDW) and stem diameter (SD).

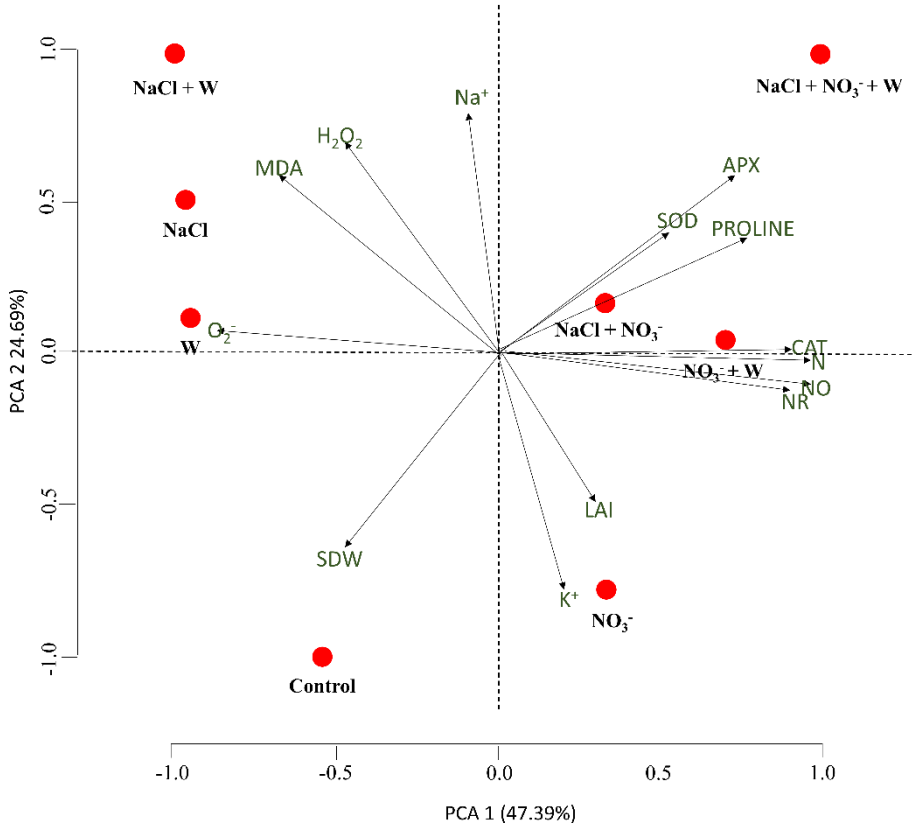


Fig. S7. Principal component analysis based on biochemical data of leaves of soybean plants treated with nitrate ($NO_3^-$) and NaCl during six days of waterlogging (W). Control plants were not exposed to waterlogging neither NaCl nor $NO_3^-$ treatments. The data used in the analysis were the activity of nitrate reductase (NR), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), the levels of $NO_3^-$, nitric oxide (NO), lipid peroxidation (MDA), superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), proline (PROLINE), sodium ($Na^+$), and potassium ($K^+$), shoot dry weight (SDW) and leaf area index (LAI).

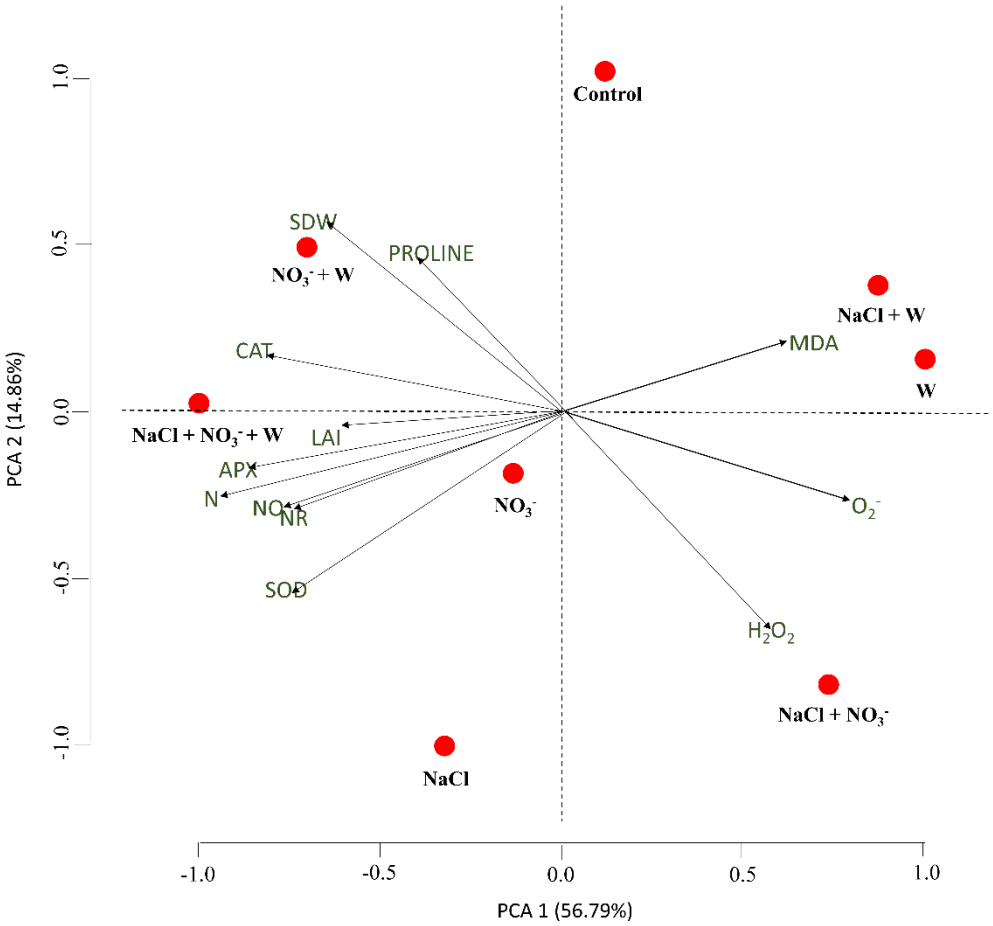


Fig. S8. Principal component analysis based on biochemical data of leaves of soybean plants treated with nitrate ($NO_3^-$) and NaCl during reoxygenation after six days of waterlogging (W). Control plants were not exposed to waterlogging neither NaCl nor $NO_3^-$ treatments. The data used in the analysis were the activity of nitrate reductase (NR), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), the levels of $NO_3^-$, nitric oxide (NO), lipid peroxidation (MDA), superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), proline (PROLINE), sodium ($Na^+$), and potassium ($K^+$), shoot dry weight (SDW) and leaf area index (LAI).