

TITLE:**AUTHOR: Rosinei Aparecida de Souza****ABSTRACT**

The commercial cropping of transgenic plants together with the use of specific herbicides and insecticides has resulted in an increasing demand for studies of environmental risk assessment. In Brazil, an additional concern over transgenic soybean crop has been to guarantee that the foreign material does not affect the biological N₂ fixation process or other benefits related to associations with soil microbes other than rhizobia. The objective of this work was to establish and to validate at a commercial cropping (Trial 1) and experimental scales (Trial 2) a set of parameters capable of assessing, under field conditions, any risks to soil microbes and/or to the biological N₂-fixation process due to the adoption of new soybean technologies. Evaluations were performed at eleven sites in six Brazilian states and in the Federal District. Analyses included quantitative parameters for soil microbes (biomass C and N – MBC and MBN; microbial respiration – MR; and microbial metabolic quotient – qCO₂) and for biological N₂ fixation (number of rhizobial cells in soil; nodule number – NN and dry weight – NDW; serological identification of rhizobial strains occupying the nodules; shoot dry weight – SDW; total N in shoot – TNS and N as ureides in shoot (TNU). Qualitative analysis of the soil bacterial community was achieved by the amplification of total soil DNA with specific primers for the ribosomal 16S gene of bacteria followed by denaturing gradient gel electrophoresis (PCR-DGGE). Results were analyzed with reference to point and temporal variability, limits of coefficient of variation (CV) and correlation among the parameters. The MBC and MR were positively related ($r=0.84$, $p\leq 0.001$, Trial 1), as well as the MBC and the MBN ($r=0.75$, $p\leq 0.001$, Trial 2). Analyses of MBC, MR and NBM were relatively easy and showed good replicability and confidence to quantitative estimation of soil microbes; the maximum acceptable CV was established as 35%. Furthermore, positive and significant correlations ($p\leq 0.001$) were obtained in Trial 2 between the following parameters: SDW and MBC ($r=0.61$) and MBN ($r=0.61$), as well as between the TNS and MBC ($r=0.65$) and MBN ($r=0.65$), indicating the viability of using the MBC and MBN parameters as bioindicators of soybean growth. It was possible to verify the homogeneity of each area, between replicates, treatments and harvests by the PCR-DGGE method. Biological N₂ fixation contributed from 72 to 88% of

the TNS and soybean growth was positively correlated with it. The parameters of NDW and SDW, with a maximum CV of 20%, were adequate for evaluation of the contribution of the biological N₂ fixation; positive correlations ($p \leq 0.001$) were obtained between SDW and TNS ($r=0.88$ and 0.96), SDW and TNU ($r=0.81$ and 0.97), as well as between TNS and TNU ($r=0.94$ and 1.00) in Trials 1 and 2, respectively. In areas with variable soil- N content or those that had received N fertilizers, the additional analyses of the TNS and TNU parameters are necessary, also with a maximum CV of 20%. The application of those parameters in Trial 2 indicated that, in the first year, there was no effect of a herbicide-transgenic soybean as well as of the composition of two herbicides in the soil microbiological parameters as well as in the biological N₂ fixation process.

Index terms: Biological nitrogen fixation, Bradyrhizobium, environmental risk assessment, Glycine max, glyphosate, microbial biomass, microbial metabolic quotient, microbial respiration, transgenic soybean.