

INTENS&FIX WP 3 TASK 10 (D10.4: Report on N transfer pathways *in situ*)

ITATINGA SITE (adapt the stem labeling technique to Acacia trees in order to study the direct N transfer in mixed species plantations)

The stem labeling technique (Swanston et al., 1998, Augusto et al., 2011, Glaser et al., 2011) was applied to appraise if this method is suitable to label acacia trees with ^{15}N , without using the dedicated organs roots or leaves. This method consist of drilling a hole into the stem of a tree while the corer is continuously sprinkled with tap water to cool the corer and mainly to avoid disruption of xylem water transport. In this hole a tube is inserted, which is connected to a bottle filled with the labeling solution. When inserting the tube, several conditions must be fulfilled as to avoid the development of air bubbles and pollution of the soil by the isotope tracer.

A first test with tap water instead of the ^{15}N label showed that the chosen *Acacia mangium* tree take up rather rapidly 200 ml within 3 hours. Stem labeling started in the morning at 10 am while the weather was sunny, air temperature was 25°C, slight wind and finished at 1 pm. Nevertheless, a single bottle system is not useful to apply a ^{15}N labeled solution according to pollution of the soil by the solution when connecting the tube with the hole. Thus a two-bottle system had been developed, tested and finally used for the labeling. The first bottle is filled with water allowing a perfect connection without bubbles of bottle and stem, a brief evaluation of the uptake of the water by the tree and the connection of a second bottle with the labeling solution without disruption of the influx. Surprisingly, the selected tree takes up no water despite excellent weather conditions. A second, dominant Acacia tree with a higher leaf biomass was selected, connected to the labeling system, water uptake was checked and finally this tree was labeled with 0.8 g of ^{15}N . Weather conditions were rather similar to our first test, except no precipitation during the two days before the labeling. Apparently, xylem water transport varies widely among trees according to physiology or stem architecture. Applying this protocol prevent any failure of the stem uptake of ^{15}N , whether the further ^{15}N dynamic in the tree is under progress.

RECOMMENDATION

- Select a dominant Acacia single stem tree, evaluate the water uptake first, and then apply the tracer using the two-bottle tracer application system.



Figure 1: stem labeling technique using a single bottle system and a two-bottle system

Leaf and root sampling scheme (more details contact Ranieri; ranieri@usp.br)

Leaves:

- from Acacia, once a day, small leaf particles from fully expanded sun crown leaves (n = 10) and three small new leaves.
- from Eucalyptus, once every two days, 10 leaves from each of the six surrounding trees.

Fine roots:

- every day from Acacia and Eucalyptus, using soil cores (diameter 5 cm, depth 0 – 10 cm) at two radial distances from the tree (25 and 75 cm) (n = 6).

Proposed collaborations (Pilot study and following plot scale study)

- Some aspects of the nitrate assimilation in the leaves (kinetics, NRA, amino acids) and the phloem transport of assimilated N (amino acids, proteins?) from the leaves to the roots or into new leaves could be treated by colleagues from UDL (Dominique Gérard, Annick Brun).
- NIRS-MIRS techniques can be used to verify that the roots belongs to Acacia and to Eucalyptus
- How to assess the length of hyphae?
- Fungal biomass can be determined using their Ergosterol concentration , but comprise ectomycorrhizal fungi and soil living fungi.