# Using maize as a reference plant material and natural $^{13}$C for field assays of soil carbon dynamics

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## Abstract

Sustainable agriculture should maintain soil organic carbon to prevent soil degradation and erosion, but soil C management still requires basic data on soil C dynamics under many climates, soil types and land uses. We applied a simple field method for the measurement of soil carbon dynamics, based on the natural $^{13}$C labelling technique of carbon inputs. The method implies the addition of locally produced maize material into the soil with C3 crops, in a simple, light and cost-effective design, and the kinetic analysis of soil $^{13}$C/$^{12}$C. In Chile the approach was applied on a nine years fertility experiment with no till conditions sustaining a wheat-oat rotation, and followed thereafter for 5 years. The experimental site is located in the Andes pre-mountain (36º55'S, 71º53'O). The soil is of volcanic origin (medial, amorphic, mesic, Typic Haploxerands) and the crop rotation wheat-oat. The labeling technique showed that a very low amount (about 1 t ha$^{-1}$) was incorporated to the soil (new C) during the time-period of the experiment (4 years). The ratio of remaining C/ added C after 4 years was very low (0.03) suggesting that the high carbon content of the soil can therefore be considered as due to a large amount of passive carbon, or to ancient carbon inputs, that have saturated the sorption capacity

## Key Words

C dynamics, no-till, volcanic soil, C4 plant residues, C crops, long-term experiment

## Introduction

Preventing soil degradation should be a central goal of sustainable agriculture, especially under intensification or colonization of new lands. The maintenance of a sufficient organic carbon content in soil surface layers is one of the major ways to prevent soil degradation. Organic carbon may act directly by improving the physical properties of the superficial layer, thus reducing soil erosion or structure destruction, and indirectly by increasing the physical and chemical fertility. The enhancement of plant growth therefore increases soil C in a positive feedback process (Tiessen *et al*., 1994). Soil management and the management of crop residues (above- and below-ground parts) is the major way of controlling soil C. For instance reduced tillage is reported to increase soil C to various extends, especially in the uppermost soil layer, which is the most sensitive to degradation (Balesdent *et al*., 2000; Dolan *et al*., 2006). In Chile, about fifty percent of the Chilean agriculture production occurs in Andisols. At present no –till management is the main practice for Chilean farmers in the Andes pre-mountains. While soil carbon dynamics and the fate of plant residues and is well documented in some countries and crop productions, there is still a need for references under many climates, soil types and land uses. To better predict the effect of the practices of C management over a wide range of situations, specific long term experiments would be required. Unfortunately, such trials cannot be implemented at each place, and would need long durations and high sensitivity, because of the dilution of added C into the existing organic matter. Undoubtly, the isotope labelling of carbon inputs is the best tool to quantify and forecast the fate of C (Coleman and Fry, 1991; Zagal, 1994). This is the conventional interest of tracers to investigate systems under dynamic regimes.

The natural $^{13}$C labelling technique (Balesdent and Mariotti, 1996) has proven to be a very powerful tool to quantify soil organic matter dynamics and trace C inputs (IAEA, 1998). It is based on the natural difference in $^{13}$C/$^{12}$C ratio between C4 plants and C3 plants. The change of vegetation from one type to the other provides a natural labelling of new carbon incorporated into the soil by the vegetation. The dynamics of soil C can be there measured by the analysis of 13C/12C in soil organic matter. The latter is determined by high-resolution stable isotope ratio mass spectrometry. The method may be applied to the analysis of total soil C, but also to organic separates to decipher processes of C transformation and analyse relevant compartments of soil organic matter. The method is very powerful but unfortunately limited to places where vegetation has changed from the C3 to the C4 type.

The objective of this study was to apply the natural $^{13}C$ technique in a volcanic soil under no-till management where production is dominated by C3 plants, by applying C4 plant material and following its fate by $^{13}C/^{12}C$ measurements, in simple controlled field experiments, as a complementary tool of soil studies, allowing for quantification ofsoil organic matter dynamics

## Methods

The experiment is located in the VIII region of south central Chile (36° 55’ S;71° 59’ W) at the Andes pre-mountains. The soil is classified as medial, amorphic, mesic, Typic Haploxerands. The climate is humid Mediterranean, with a precipitation over 1400 mm. Mean temperature is between 12,5° and 13,9°C (Del Pozo, 1999.)

The experiment has a completely randomized block design with 3 replicates. It is a long-term no till experiment (at present thirteen years old) with different sources of N and P fertilizer; with a crop rotation wheat-oat, and with the following treatments: T1. Control (no nitrogen), only triple superphosphate (TSF). T2. TSF + Chilean Salpeter. T3. Urea + Ammonium mono phosphate (MAF). T4. Urea + MAF + 500 kg $ha^{-1}$ $CaCO_3$. T5. Urea + MAF +1000 kg $ha^{-1}$ $CaCO_3$. Nitrogen rate is 150 kg $ha^{-1}$ (applied in two occasions); P rate is 150 kg $ha^{-1}$ (as $P_20_5$); K rate 120 kg $ha^{-1}$ (as $K_20$).

Maize residues were applied in the main no tillage experiment, establishing 4 $m^2$ specific sub-plots (isotope reference plots). To develop the methodology measurements were concentrated on treatments TSF + Chilean Salpeter and Urea + MAF +1000 kg $ha^{-1}$ $CaCO_{3.}$ The main plots (fertilizer treatments) with C3 crop are used to measure unlabelled isotopic composition (these plots are analyzed for the C and $^{13}C$ only). The sub plots are geo-referenced and staked. The material used was leaves and stems harvested by hand (600 $m^2$), cut in cm pieces and air dried at existing outside conditions. Moisture content was determined before application and a sub-sample taken for C and $^{13}C$ analysis. Before maize residues application, C3 crop residues were a removed. Due to the high content in organic matter (8-10%) high amounts of maize residues were applied in only 4 $m^2$ (e.g. 7 kg $m-^2$) in one or two more operations. Maize C is incorporated every two years at the same place. This is a cumulative labelled-C addition, for a better sensitivity and time-integration of processes. Plots should be followed at least 5 years at the yearly time-step, considering that the first year is establishment time.

The soil is typically analyzed before crop return and the new maize addition. Soil samples are taken in one $m^2$ at the center of the subplots. One quarter is used each year, an properly marked, in order to provide an unbiased and representative estimate of soil C and residue C. Samples were dried and completely reduced to a size finer than 0.2 mm by a step-by step reduction of sample aliquots. Organic C and delta$^{13}C$ were analyzed by EA-IRMS in a specialized laboratory (University of Ghent, Faculty of Bioscience Engineering, ISOFYS laboratory). Soil bulk density has been measured. Twenty four (24) measurements (3 replicated blocks x 4 years x labelled+unlabelled) is the minimum set of measurements per treatment.

The proportion *f* of residue C in soil C will be determined as

$$f = (\mathrm{delta}^{13}C_{\mathrm{labelled\_plot}} - \mathrm{delta}^{13}C_{\mathrm{unlabelled\_plot}}) / (\mathrm{delta}^{13}C_{\mathrm{maize}} - \mathrm{delta}^{13}C_{\mathrm{crop}})$$

The amount of residue-C remaining is:

$C_{\mathrm{residue}} = C \times f$, where C is the density of soil C (t/ha)

Carbon, N and delta$^{13}C$ content of maize DM were also determined by using EA-IRMS. Crop yields were recorded during the experiment. The change in stable carbon isotope signature was interpreted as follows: the delta$^{13}C$ of reference plots under C3 plants was considered as unaffected by time and the values for all years were pooled, providing n=24 independent measurements for each horizon. Two outlier data were removed from the dataset. The delta$^{13}C$ of labelled plots was considered as dependent on time only and n=6 independent values of replicates plots were used for each year. This enabled to calculate the 95% confidence interval on the difference between labelled and unlabelled plots, with a comfortable number of degrees of freedom.

## Result

Table 1 shows unlabeled and labelled total C contents and delta $^{13}C$ values in the soil. The results indicate the very high content of total C in this volcanic soil, characteristic that dilutes the input of new C. Despite of this effect an increase in carbon isotopic signature could be measured.

**Table 1. Unlabeled and labeled total C contents and delta $^{13}$C values in the soil (n=24).**

| Soil depth (cm) | Unlabeled total C (t/ha) | Labeled Total C (t/ha) | Unlabeled delta $^{13}$C (‰) | Labeled delta $^{13}$C (‰) |
|---|---|---|---|---|
| 0 to 5 | 31,14 | 33,23 | -26,61 | -26,40 |
| SD | (3,67) | (2,18) | (0,34) | (0,28) |
| 5 to 10 | 28,27 | 29,05 | -26,32 | -26,23 |
| SD | (3,12) | (2,64) | (0,38) | (0,39) |
| 10 to 20 | 40,89 | 40,22 | -25,79 | -25,76 |
| SD | (7,05) | (6,82) | (0,30) | (0,30) |
| 20 to 40 | 58,67 | 54,24 | -25,17 | -25,11 |
| SD | (15,02) | (11,72) | (0,37) | (0,31) |

In fact, following the interpretation described in the section Methods, the signature demonstrated a progressive trend versus time in the 0-5 cm in accordance with the, whereas the amount of maize-derived-C in the horizons below was much smaller, and not significant below 10 cm (Figure 1).

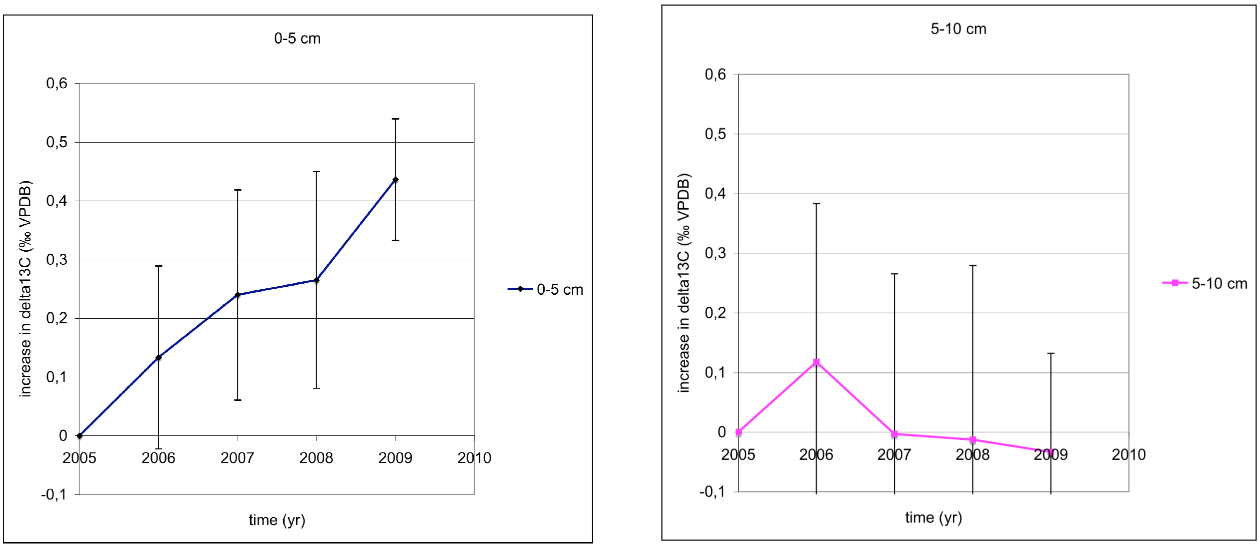

**Figure 1. Mean and 95% confidence interval on the difference in delta$^{13}$C between maize-labelled and unlabelled plots.**

This enabled to estimate minimum and maximum estimates of the amount of maize-derived carbon in the soil. Table 2 provides this estimate at the end of the experiment in 2009
The increase in delta $^{13}$C is relatively low to the very high dilution in the large pool of preexisting organic matter (100 t C ha$^{-1}$ in the top 20 cm; Table 1).

This amount can be related to the cumulated amount of added carbon residues (29 t C ha$^{-1}$).
The ratio of remaining C/ added C after 4 years is 0.97/29 = 0.033 and is lower than (<0.2). This conventional ratio means that plant material decays as rapidly in this type of soil as in other soils. The high carbon content of the soil can therefore be considered as due to a large amount of passive carbon, or to ancient carbon inputs, that have saturated the sorption capacity.
Further interpretation will be done by using the RothC model (Jenkinson and Rayner, 1977) of soil organic carbon dynamics, and the need for a specific calibration in this type of soil will be evaluated. The study also demonstrated that with appropriate experimental design, methods of sampling and analysis, a confidence interval as low as ±0.15 ‰ VPDB could be obtained on the change in delta$^{13}$C of the bulk soil.

**Table 2. Contribution from maize residue to soil organic carbon (4 years).**

| | **Maize-derived C (t $ha^{-1}$) in 2009** | | |
|---|---|---|---|
| | minimum (conf. 95%) | mean | maximum (conf. 95%) |
| 0 to 5 cm | 0,74 | 0,97 | 1,21 |
| 5 to 10 cm | 0,00 | 0 | 0,26 |
| 10 to 20 cm | 0,00 | 0 | 0,12 |
| 20 to 40 cm | 0,00 | 0 | 0,00 |
| | | | |
| 0 to 10 cm | 0,74 | **0,97** | 1,46 |
| 0 to 40 cm | 0,74 | **0,97** | 1,58 |

## Conclusion

The labeling technique showed that a very low amount was incorporated to the soil (new C) during the time-period of the experiment (4 years) and the ratio of remaining C / added C was very low suggesting that the high carbon content of the soil can therefore be considered as due to a large amount of passive carbon, or to ancient carbon inputs, that have saturated the sorption capacity.

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## References

Balesdent J, Mariotti A (1996) Measurement of soil organic matter turnover using $^{13}C$ natural abundances. In Mass Spectrometry of Soils (Eds T.W. Boutton & S.I. Yamasaki.) pp 83-111. (Marcel Dekker Inc., New York)

Balesdent J., Chenu C, Balabane M (2000) Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Tillage Research,* **53**, 215-230.

Coleman DC, Fry B (1991) Carbon and isotopes techniques. Academic Press, San Diego

Dolan, MS, Clapp CE. Allmaras RR, Baker JM, Molina JAE (2006) Soil organic carbon and nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management. *Soil and Tillage Research.* **89**: 221-231.

Jenkinson D.S, Rayner JH (1977) The turnover of soil organic matter in some of the Rothamsted classical experiments. *Soil Science* **123**:298-305.

IAEA. International Symposium on Isotope Techniques in the Study of Past and Current Environmental Changes in the Hydrosphere and the Atmosphere, Vienna(1998) Proceedings. (IAEA-SM-349/46).

Tiessen H, Cuevas E, Chacon P (1994) The role of soil organic matter in sustaining soil fertility. *Nature* **371**: 783-785.

Zagal, E (1994) Carbon distribution and nitrogen partitioning in a soil-plant system with barley (*Hordeum vulgare* L.), ryegrass(*Lolium perenne*) and rape (*Brassica napus* L.) grown in a$^{14}CO_2$ atmosphere. *Plant and Soil* **166**, 63-74.