

## COMBINING AGGREGATE FRACTIONATION AND MOLECULAR ANALYSES TO UNREVEAL LAND USE EFFECTS ON SOIL CARBON STORAGE DYNAMICS

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### Introduction

Carbon storage in soil has been proposed as one of the most effective mitigation strategies to counteract climate change by reducing global CO<sub>2</sub> emissions. Sustainable land-uses, such as the implementation of temporary (ley) grassland, may provide several ecosystem services (Lemaire et al., 2015). Many of those services are mediated by an increase of soil organic matter (SOM) content, which constitutes the keystone for these purposes. However, carbon storage is not only a matter of quantity, since the stability of new pools at long-term must be taken into consideration. Inputs of labile carbon may stimulate the so-called priming effect, resulting in an increased turnover of older soil C (Kuziakov, 2010). Consequently, researches aim to precise the nature of the newly stored pool and its vulnerability to loss. Nevertheless, the evaluation of stability and fluxes of C pools constitutes a considerable challenge, since soil is composed by a complex and variable matrix of three phases in which biotic communities maintain a wide range of interactions with abiotic factors. Thus, a molecular characterization of SOM through a multidisciplinary approach is necessary. The aim of the present work is to investigate SOM from ley grassland (LG) plots at a molecular level, using three different land uses as controls: continuous grassland, continuous cropland, and bare fallow. The working hypothesis affirms that LG may improve the net C storage at long-term, changing the dynamics of SOM accrual. A combination of soil fractionation, C3 to C4 vegetation switches, stable isotope (SI) analyses, compound specific analyses on lignin biomarkers, and <sup>13</sup>C solid state nuclear magnetic resonance (NMR) were used to test the hypothesis.

### Material and methods

Soil samples were collected at the experimental observatory established in 2005 in Lusignan (<http://www.soere-acbb.com/>). Ley grassland comprised a rotation of 6 years grassland followed by 3 years of maize. An *in situ* labelling using maize as selected C4 crop, was carried out to follow-up the incorporation of newly-derived SOM. To overcome spatial variability, a decomposition of soil mosaic into smaller “pieces” by aggregate and density fractionation was carried out. Successively, lignin-derived phenols, considered as biomarkers of vegetal inputs, were extracted from soil samples with the CuO oxidation method and then analysed by GC coupled with SI spectrometer. Finally, shifts in molecular composition of particulate organic matter (POM) were evaluated by solid state NMR, using a cross polarization single pulse (CPSP) sequence and two different molecular mixing models.

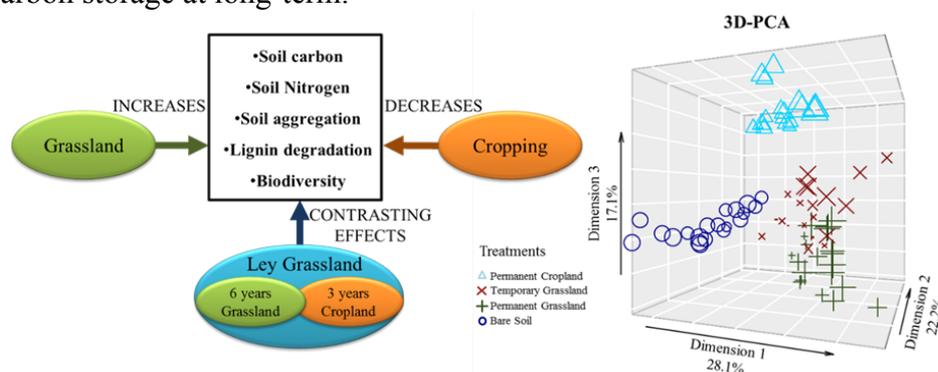
### Results and discussion

LG had intermediate C contents between permanent grassland and permanent cropland. No significant increases of C contents were found for bulk samples of LG in the arable layer (0-30 cm) when compared with permanent cropland, but LG and permanent grassland had a similar aggregation profile and C distribution. Data obtained from lignin biomarkers, SI, and NMR confirmed that LG maintained a grassland “footprint” after 3 years of continuous maize cropping, as summarized in figure 1. The reactivity of soil aggregate fractions to changes of land use decreased with aggregate size; indeed, very low percentages of C4-SOM were found

for the finer fraction of LG. The types of vegetal input to soil (maize vs. grass) and the above to belowground biomass ratios controlled C as well as lignin dynamics. The impact of plant species and tillage practices is often underestimated when soil C stocks are evaluated. Our data suggest that these variables must be considered for long-term stability of C, even when total C stocks are not yet subjected to significant losses. For example, lignin turnover was monomer and aggregate dependent, different patterns were found for each land use, but no selective lignin preservation was highlighted. Maize crops returned higher lignin amounts to soil, mostly from aboveground biomass, but no lignin content increases were found if compared with grassland. Thus, the persistence of lignin may be influenced by tillage characteristics and it is related to its interaction within the soil matrix and the nature of vegetal inputs, rather than to its intrinsic recalcitrance. Furthermore, soil C from finer soil fractions of LG had slower C-turnover, when compared with corresponding samples under continuous maize. After the 3 years of maize cropping, grassland was newly sown and soil samples were collected 2 years later to evaluate the treatment effect on the microbial biomass. For LG soils, microbial communities reacted quickly to land use change and lost most of their C<sub>4</sub>-derived signature, possibly due to a preferential degradation pattern for the grassland-derived SOM, developed during the 6 years under grassland, and maintained during the 3 years of maize cropping. According to these data, NMR spectra highlighted a higher contribution of alkyl-C in fine fraction of LG, in which the older-C (grassland derived-C) is stored. Alkyl-C may indicate a preservation of grassland vegetal material and/or a microbial proliferation in this fraction, implying a preference of microbial communities for the grassland C rather than for newly derived maize C.

## Conclusions

The study of different carbon pools highlighted how ley grassland rotation tends to maintain a grassland footprint even after several years of maize cultivation. In a context of land use change, the use of aggregate fractionation, SI, NMR, and compound specific analyses allowed to identify this legacy effect, and to unveil some of its underlying mechanisms which may promote carbon storage at long-term.



**Figure 1.** A schematic representation (left) of LG systems. Principal Component Analysis plot (right) of the soil organic matter “footprint” under different land-uses (Panettieri et al., 2017).

## References

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