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# Investigating the impact of plant composting on soil organic matter and microbial community dynamics using stable isotopes and molecular analyses

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## Abstract

Composts are often applied on arable soils to sustain the content in soil organic matter (SOM). The mechanisms leading to an increase of SOM content are still poorly understood. Our objectives were to evaluate the effect of composting on the dynamics of C and N in a soil and to investigate the potential relationships with the chemical composition, the structure of the soil microbial degrader communities and the aggregate stability. To this aim, we used <sup>13</sup>C and <sup>15</sup>N labelled plant materials composted or not which were incubated in an arable soil during 3 years. Our results showed that composting plant material had an impact both on C and N dynamics in soil. A significant but transient impact on the microbial degrader community structure and the aggregate stability was also observed. These changes in microbial activity can be related to the initial differences in the chemical composition of the plant residues due to composting.

## Introduction

Loss of soil organic matter (SOM) may lead to reduced soil fertility and increased soil erosion. A small but consistent increase in SOM could mitigate climate change effects by storing atmospheric CO<sub>2</sub>-C [1]. Annual application of exogenous organic matter (EOM) to cultivated land may lead to long continued accumulation of SOC [2]. Composts are often applied on arable soils to sustain the content in SOM but the mechanisms leading to an increase of SOM content are still poorly understood. Our objectives were to better understand the effect of composting on the dynamics of C and N in soils and to investigate the potential relationships with the chemical composition, the structure of the soil microbial degrader communities and the aggregate stability.

## Material and Methods

### *Plant material*

In this study, we used <sup>13</sup>C and <sup>15</sup>N labelled plant materials composted (12 weeks) or not. The C content were 35% and 39% and the C/N ratio 7.7 and 11.3 for composted (COMPO) and non-composted (NO-COMPO) plant residues, respectively. The <sup>13</sup>C signatures were 271‰ and 263‰ and the <sup>15</sup>N signatures were 1064‰ and 1473‰, respectively for COMPO and NO-COMPO plant residues. The relative amount of lignin was measured by the CuO oxydation method [3] and the amount of non-cellulosic sugar by the acetate alditol method [4].

### *Soil incubation set up*

Soil samples (Typic Hapludalf) were obtained from the surface layer (0-28cm) of an experimental plot located at Feucherolles (France). The soil is a silt loam (7% sand, 76% silt, 17% clay), with 8.9g kg<sup>-1</sup> of total organic carbon (TOC), 1.1 g kg<sup>-1</sup> of nitrogen, and a pH of 6.9. The bulk isotopic signature of the organic carbon was -24.8±0.2 ‰. After being collected, the soil was sieved moist to < 5 mm. Free roots and plant debris were discarded during the sieving. Prior to use, soil samples were preincubated

at 60% WHC for 3 weeks at 20°C. Soil samples (125 g dry weight equivalent) were put in serum bottles with Teflon® rubber stoppers crimped on with aluminium seals and amended either with 360 mg of composted (COMPO) or 330 mg of non-composted plant residues (NO-COMPO), corresponding to a C addition of 11.5% of the TOC. Control samples did not received any amendment. Every samples were adjusted to 80% WHC. Then, the sample bottles were flushed with CO<sub>2</sub> free air and incubated at 20°C in the dark for 3 years.

### *<sup>13</sup>C and <sup>15</sup>N measurement*

Throughout the experiment, <sup>13</sup>C and <sup>15</sup>N concentrations were analysed in the bulk soil, the mineralised fraction (CO<sub>2</sub> or nitrates) and the microbial biomass (fumigation extraction technique) using EA-IRMS. The amount of <sup>13</sup>C or <sup>15</sup>N measured in each fraction was then expressed in percentage of the initial amount for COMPO and NO-COMPO samples, respectively. The evolution of the chemical composition of plant residues was determined by <sup>13</sup>C-NMR after HF treatment [5] after 6, 12 and 36 months of incubation. The soil microbial communities' degraders of labelled plant residues were followed using PLFA-SIP approach [6] performed on a GC-c-IRMS. Comparison of the total and labelled-microbial communities was done using the Evenness index.

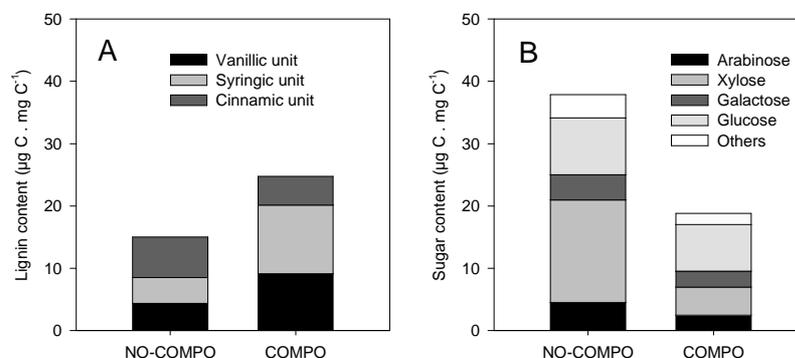
### *Soil physical analysis*

The aggregate stability of samples taken after 45 days and 3 years of incubation was determined using the slow wetting test of Le Bissonais [7]. Briefly, 5 grams of calibrated aggregates (3.15-5 mm) were put on a filter paper on a tension table at a matrix potential of -0.3 kPa for 60 min; aggregates were then transferred to a 50-µm sieve previously immersed in ethanol for the measurement of fragment size distribution. The 50-µm sieve previously immersed in ethanol was gently moved 5 times with a Henin apparatus to separate fragments <50 µm from those >50 µm. Second, the >50 µm fraction was collected from the 50-µm sieve, oven-dried and gently dry-sieved by hand on a column of six sieves: 2000, 1000, 500, 200, 100 and 50 µm. The mass percentage of each size fraction was then calculated; the fraction < 50 µm is the difference between initial mass and the sum of the six other fractions. The aggregate mean weight diameter (MWD) for each sample was also calculated.

## **Results**

### *Change in chemical composition after composting*

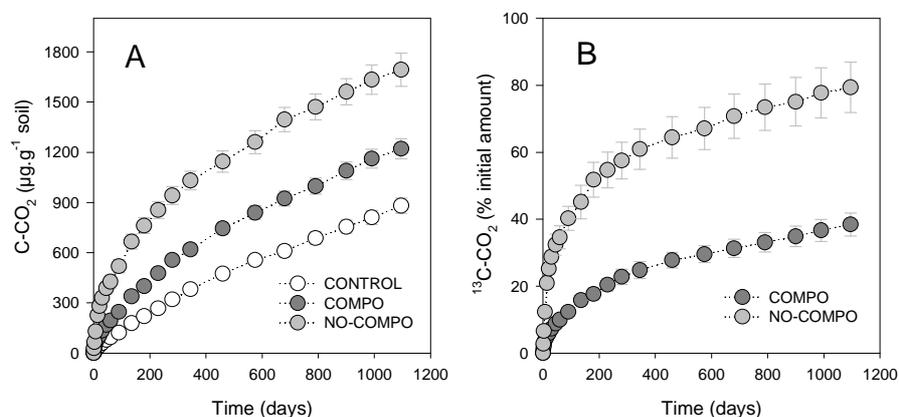
Chemical analyses of plant material by GC-MS reveals that the lignin content increased by 70% and the ratio of cinnamic unit / vanillic unit decreased, suggesting a preferential degradation upon composting (Figure 1A) . The composting process also decreased the content of sugars by 50% (Figure 1B). The ratio of C6 / C5 sugars (glucose+galactose / arabinose+xylose) increased, suggesting a higher contribution of microbial over vegetal derived carbohydrates.



**Figure 1: Comparison of the lignin content (A) and non-cellulosic sugar content (B) in the non composted (NO-COMPO) and composted (COMPO) plant residues. Data are the mean of three replicates.**

### Effect of composting on C and N dynamics

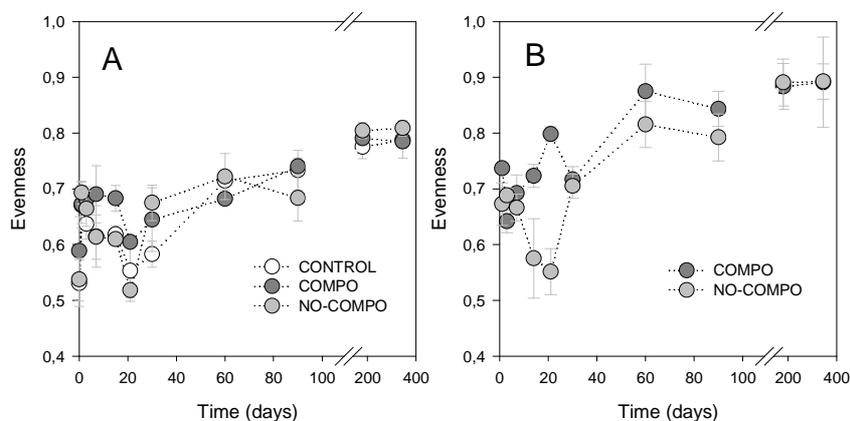
The C mineralisation of non-composted materials (Figure 2), and the priming effect of SOM (data not shown) were higher than those observed for composted residues. Thus, 3 years after the amendments in soil, the loss of C due to composting process (about 70%) was compensated. The N mineralisation of non-composted plant residues was lower than composted ones during the first year, due to higher amount of N assimilated into the microbial biomass (data not shown).



**Figure 2: Evolution of the cumulative total CO<sub>2</sub> (A) and <sup>13</sup>C-CO<sub>2</sub> evolved relative to the initial amount of composted or non composted plant residues (B). Standard deviations correspond to three replicates.**

### Change in microbial community structure

In plant residues amended soil, GC-c-IRMS analysis revealed that 21 PLFA were significantly <sup>13</sup>C-enriched compared to the control soil (C11:0, C12:0, C13:0, C14:0, iC15:0, aC15:0, C15:0, iC16:0, C16:1(9)cis, C16:0, iC17:0, cycC17:0, C17:0, C18:2(9,12), C18:1(9)cis, C18:1(9)trans, C18:0, cycC19:0, C19:0). From 3 to 30 days of incubation, the total amount of <sup>13</sup>C incorporated into PLFA was higher for the non-composted material than in composted one, which was more stable over the incubation (data not shown). The distribution of <sup>13</sup>C into the PLFA was more even in the composted plant residues amended soil during the first 30 days, suggesting a less specific population of microbial degraders than in the non-composted plant residues amended soil (Figure 3). Thereafter, no difference between the two treatments was observed.



**Figure 3: Evolution of the evenness index for all treatments calculated from total PLFA (A) and <sup>13</sup>C-PLFA (B) during one year of incubation. Standard deviations of correspond to 3 replicates of sampling dates.**

The comparison of the % of change in the CO<sub>2</sub> rate and that of the <sup>13</sup>C-PLFA for both treatments revealed that the level of activity of the microbial degraders is highly related ( $r^2=0.86$ ,  $P<0.005$ ) to their structure throughout the incubation. This result has to be related to the evolution of the chemical structure of the plant residues showed by <sup>13</sup>C-NMR spectra.

#### *Evolution of the organic matter composition*

<sup>13</sup>C-NMR analysis showed differences in the spectra between composted and non-composted plant residues (data not shown). Alkyl (-10 to 45ppm), aryl (110-160 ppm) and carboxylic (160-220 ppm) carbon contents were relatively more important after composting. After 6 months of incubation in the soil, the composition of composted and non-composted plant residues were much more similar, with an increase of O-alkyl (45 to 110 ppm) and aryl carbon contents, indicating higher aromaticity. There was no major change from 6 months onwards.

#### *Effect of amendments on soil aggregate stability*

The addition of both amendments increased soil aggregate stability during the first months of the incubation (45 days), without differences between composted and non-composted residues. However, this aggregating effect did not last, and by the end of the incubation (three years), the aggregate stability of the amended soils was not different from that of the non-amended soil.

### **Conclusion and perspectives**

This study shows that composting plant material had an impact both on C and N dynamics in soil. Composting plant material had a significant but transient impact on the microbial degrader community structure when applied to a soil. This result could explain the dynamics of aggregate stability. The amount and the relative specificity of the microbial degraders' communities were higher in the non-composted plant residues treatment only during the first month of incubation, which can be related to the initial differences in chemical composition of the plant residues.

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