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Short-term interaction between organic matter from biofuel defatted seed cakes and soil microbiota in two intensive horticulture systems

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ABSTRACT

In the last decades, use of biodiesel, produced by oil seeds, has widely increased. Defatted seed cakes are the most important byproducts of biodiesel production chain and their agricultural valorization is becoming a global challenge. The aim of this study was to characterize carbon molecular composition of several seed cakes (*Brassica carinata* A. Braun, *Brassica napus* L. and several components of *Helianthus annuus* L. seed wastes) by means of ^{13}C CPMAS-NMR spectroscopy and evaluate their short-term effects on soil microbial activities, in two intensive horticulture systems. Defatted seed cakes used in this study have relevant differences in chemical composition and significant conditioning effects on soil microbiota. Seed cakes from sunflower were characterized by a higher content of celluloses, as carbohydrates composition, while *Brassicaceae* seed cakes were rich in more biochemical stable compounds and secondary metabolites, such as glucosinolates precursors of anti-microbial molecules. These latter ones, along with the larger amount of carbon molecules with an highest biochemical stability, negatively affected microbial growth resulting, generally, in lower soil activities. In conclusion, this study unraveled, for the first time, the molecular carbon composition of several biofuel byproducts and showed how the seed cakes can be evaluate for soil amendments, thus permitting to convert byproducts from biofuel chain to co-products useful in agriculture.

1. Introduction

One of the major challenges of the 21st century is the sustainable growth of population that must be achieved by reducing and limiting environmental footprint. To contrast greenhouse gas emissions and decrease the reliance on crude oil fuels, developed countries promoted alternative fuel use, such as biodiesel. Biodiesel is commonly produced by transesterification of vegetable oil, extracted from oil seeds, as soybean, palm, sunflower and rapeseed. The most important by-products resulting from oil extraction are represented by defatted seed cakes, with 30 Mt produced only in the 2015 in the European Union [1]: therefore, their disposal and evaluation as co-products, is a global challenge. Normally, seed meals are tested using animal feeding trials, except for meals from *Brassicaceae* seeds, due to their glucosinolates content that reduces palatability [2]. Conversely, these latter ones, when are used as organic amendments in agriculture, can be considered as an effective tool to control soil-borne diseases, because of the release of toxic secondary metabolites [3].

Biochemical soil properties are considered good soil quality indicators, because of their quick responses and correlation to the soil organic carbon, providing indications on quantitative/qualitative changes of organic matter [4]. Dehydrogenase and hydrolase activities are usually related to the presence of viable microorganisms and their oxidative capability [5]. Among hydrolytic enzymes, phosphomonoesterase and β-glucosidase activities have been frequently used, since they catalyse important carbon and organic phosphorus reactions into the soil [6]. Urease activity is considered to be important in nitrogen cycling, because involved in the urea decomposition [7].

^{13}C Cross Polarization Magic Angle Spinning (CPMAS) Nuclear Magnetic Resonance (NMR) spectroscopy is useful to provide a description of the total organic chemical carbon composition of complex matrices [8,9]. The chemical shifts of different C atoms is strictly related to their molecular environment, allowing the attribution of observed carbons to a particular class of organic compounds and getting important information about their chemical type and the nature and number of substituents. To the best of our knowledge, none study is focused on the chemical carbon composition of biodiesel byproducts, using ^{13}C CPMAS-NMR spectroscopy.

While the effects of organic amendments on soil quality status by means of microbial activity and soil enzymes have been widely investigated [6,10], to the best of our knowledge the use of biofuel chain byproducts is a topic still poorly investigated, especially in intensive

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horticulture systems [5,11], and none study reports a clear description of the their molecular carbon composition. Therefore, in this study, defatted seed cakes and seed waste from several species were characterized by ^{13}C CPMAS-NMR and their effects, as organic amendments, were evaluated for two years in two agricultural systems, greenhouse and open field, to prove the hypothesis that the application of seed cakes will be able to either i) to increase microbial activity and stimulate soil enzymatic activities or ii) in the case of *Brassicaceae* seed cakes, affect soil microorganisms with the release of glucosinolate metabolites.

2. Material and methods

2.1. Experimental design, organic amendments, and soil sampling

The study was performed in two experimental sites located in Southern Italy, Campania region, (40° 35′ 10.723″ N, 14° 56′ 52.166″ E, and 40° 35′ 3.732″ N, 14° 58′ 55.297″ E, respectively), under greenhouse and open field conditions. Greenhouse field was a clay loam soil, large EC (0.37 ± 0.08 dS m^{-1}), low organic carbon content (17.0 ± 0.5 g kg^{-1}) and a good total nitrogen content (1.6 ± 0.1 g kg^{-1}). Open field system was a clay loam soil, with about 9.98 g kg^{-1} of total organic carbon, 0.8 g kg^{-1} of total nitrogen and a low EC (0.07 ± 0.01 dS m^{-1}). During the trial, chard (*Beta vulgaris* subsp. *cicla* L.) and tomato (*Solanum lycopersicum* L. cv. Auspicio) plants were cultivated in the greenhouse and in the open field, respectively. Defatted seed cakes deriving from an experimental oil extraction system, were used as organic treatments: BN, rapeseed (*Brassica napus* L., total N 45 g kg^{-1}); BC, Ethiopian mustard (*Brassica carinata* A. Braun, total N 50 g kg^{-1}); SunD, from partially decorticated sunflower seeds (total N 50 g kg^{-1}); SunF, from sunflower flakes and pelletized seed meals (total N 38 g kg^{-1}); SunT, from seed teguments of sunflower (*Helianthus annuus* L., total N 14 g kg^{-1}). Not treated (CNT) and a mineral fertilized (MIN) plots were also defined. For both systems 21 plots, seven treatments in triplicate, were randomized. All seed cakes were supplied at doses of 130 and 150 kg ha^{-1} of nitrogen, in greenhouse and open field system, respectively. In MIN plots, a similar amount of nitrogen, as commercial mineral fertilizer, was added. Experimental study was conducted for two years, 2012 and 2013, with one amendment *per* year, in March for the greenhouse system and in May for the open field. Soil sampling was carried out after one month for the greenhouse system and after 1 and 3 months for the open field system. One sample from each plot was composed by five mixed subsamples W scheme collected, within rows, from the topsoil (0–20 cm). Before carrying out any analysis, all soils were sieved (< 2 mm) and stored at 4 °C.

2.2. ^{13}C CPMAS-NMR of defatted seed cakes, soil enzymatic activities and catabolic profile

^{13}C CPMAS NMR spectroscopy was performed by CERMANU-Interdepartmental Research Centre for Nuclear Magnetic Resonance, University of Napoli Federico II. Briefly, experiments were carried out on a Bruker AV-300 equipped with a 4 mm wide-bore MAS probe, with the following acquisition parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; ^{1}H-power for CP 92.16 W: ^{1}H 90° pulse 2.85 μs; ^{13}C power for CP 150,4 W; 1 ms of contact time; 30 ms of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps. The cross polarization pulse sequence was applied with a composite shaped “ramp” pulse on the ^{1}H channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The Fourier transform was performed with 4k data point and an exponential apodization of 100 Hz of line broadening. For the interpretation of ^{13}C-CPMAS-NMR spectra, the overall chemical shift range was divided into the following main resonance regions: alkyl-C (0–45 ppm); methoxyl-C and N-alkyl-C (46–60 ppm); O-alkyl-C (61–110 ppm); unsubstituted and alkyl-substituted aromatic-C (111–145 ppm); O- substituted aromatic-C (146–160 ppm); carboxyl- and carbonyl- C (161–190 ppm). To estimate the contribution of specific functional, the area of each spectral region (Riabs) was divided by the sum of all spectral areas, to obtain a relative amount (MestreNova 6.2.0 software, Mestre-lab Research, 2010): Ri % = (Riabs/ΣRiabs) x 100.

In order to highlight the specific molecular characteristics of different organic materials, the following structural indexes were derived from the calculated C distribution over the NMR spectra [12]:

- the Hydrophobic index is the ratio of signal intensities found in chemical shift intervals for apolar alkyl and aromatic C components over those of hydrophilic C molecules

$$HB = \Sigma[(0–45)+(45–60)/2+(100–160)] \,/\Sigma\, [(45–60)/2+(60–110+(160–190)]$$

- the Alkyl ratio compare the abundance of relative areas of apolar alkyl components with that of hydrophilic O-alkyl-C molecules

$$A/OA = [(0–45)] \,/[(61–110)]$$

- the Lignin ratio, relates the abundance of Methoxyl-C + N-alkyl groups to that of O-aryl-C

$$LR = (46–60) \,/\, (146–160)$$

Dehydrogenase (DHY, E.C. 1.1) and urease (UR, E.C. 3.5.1.5) activities were assayed using triphenyltetrazolium chloride and urea as substrates, respectively, according to Scotti et al. [13]. Alkaline phosphomonoesterase (PHO, E.C. 3.1.3.2) and β-glucosidase (GLU, E.C. 3.2.1.21) activities were determined with *p*-nitrophenyl phosphate (*p*-NPP) or *p*-nitrophenyl-b-D-glucopyranoside (*p*-NG), respectively, as substrates. Specific buffers, pHs, and reaction stop procedures were used as reported in Scotti et al. [13]. Total hydrolytic activity was assessed by the fluorescein diacetate method (FDA) [14]. Triplicate analysis were performed for each activity assay.

Microbial catabolic profile was evaluated by BIOLOG EcoPlatesTM method based on carbon substrate utilization, as previously described by Scotti et al. [6]. Average well colour development (AWCD) was calculated as the sum of activities measured in all wells of each plate, divided by the 31 carbon sources. Shannon's index was calculated as H’ = Σ*pi* ln *pi*, where *pi* is the ratio of the activity on a particular substrate to the sum of activities on all substrates [15,16].

2.3. Statistical analyses

Data were analyzed by one-way ANOVA, by considering each type of amendment as an independent variable, and the Student's t-test for simple pair-wise comparisons. The homogeneity of variances and normality of distribution were tested with the Levene's and Kolmogorov–Smirnov tests, respectively. The relationship between soil biochemical properties and relative distribution of the ^{13}C-CPMAS-NMR of seed cakes, was assessed by using Pearson correlation coefficients. Significance was evaluated in all cases at $P < 0.01$. Statistical analyses were carried out using JMP 8 Software [17].

3. Results

3.1. Chemical composition of seed cakes

The ^{13}C CPMAS NMR spectra of organic biomasses were characterized by strong signals in the O-alkyl-C interval (Fig. 1), revealing a large content of polysaccharides and carbohydrates, which accounted