Soil Biology & Biochemistry 56 (2013) 40–48

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Litter quality assessed by solid state ^{13}C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios

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ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form
17 February 2012
Accepted 6 March 2012
Available online 29 March 2012

Keywords:
C-cycle
C stocks
Decomposition
Litterbag
Litter quality
Principal component regression
Proximate cellulose and lignin

ABSTRACT

Predictions of litter decomposition rates are critical for modelling biogeochemical cycling in terrestrial ecosystems and forecasting organic carbon and nutrient stock balances. Litter quality, besides climatic conditions, is recognized as a main factor affecting decay rates and it has been traditionally assessed by the C/N and lignin/N ratios of undecomposed materials. Here, solid state ^{13}C NMR spectroscopy and proximate chemical analysis have been used to characterize litter organic C in a litterbag experiment with 64 different litter types decomposing under controlled conditions of temperature and water content. A statistical comparative analysis provided evidence that C/N and lignin/N ratios, showing different trends of correlation with decay rates at different decomposition stages, can be used to describe the quality of undecomposed litter, but are unable to predict mass loss of already decomposed materials. A principal component regression (PCR) model based on ^{13}C NMR spectra, fitted and cross-validated by using either two randomly selected sets of litter types, showed highly fitting predictions of observed decay rates throughout the decomposition process. The simple ratio 70–75/52–57 corresponding to O-alkyl C of carbohydrates and methoxyl C of lignin, respectively, showed the highest correlation with decay rate among different tested parameters. These findings enhance our understanding of litter quality, and improve our ability to predict decomposition dynamics. The ^{13}C NMR-based 70–75/52–57 ratio is proposed as an alternative to C/N and lignin/N ratios for application in experimental and modelling work.

1. Introduction

Leaf litter decomposition is a critical process for biogeochemical cycling in terrestrial ecosystems (Attiwill and Adams, 1993) with slow decomposition rates enhancing the accumulation of organic carbon and nutrient stocks. Litter decomposition rates are strongly affected by climatic variables (Aerts, 1997) and litter quality (i.e. the susceptibility of the substrate to decomposer attack; Swift et al., 1979; Rovira and Vallejo, 2007), which interact and prevail across different spatial scales (Liski et al., 2003). Temperature and water availability are considered the most important factors acting at global and regional scale (Aerts, 1997), while at local scale, with nearly uniform climate, litter decay rate is mostly affected by litter quality (Meentemeyer, 1978).

The definition of litter quality in terms of organic chemical composition (Swift et al., 1979) is operationally difficult because litter material hold a multitude of organic compounds with different susceptibility to decomposition (e.g. lignin, tannins, cellulose, organic acids, aminoacids, simple sugars) whose relative fractions, together with several inorganic elements (e.g. N, P, S), vary with the decay stage (Berg and McClaugherty, 2008). During the last decades a substantial effort has been made in search of effective indicators of litter quality, capable to provide reliable predictions of litter decay rate (Cornelissen and Thompson, 1997; Cornwell et al., 2008). Traditional approaches have been based on the assessment of selected litter characteristics to identify parameters or indexes correlated with decay rates, and thus useful for predictive purposes (Meentemeyer, 1978; Melillo et al., 1982; Coq et al., 2010). Several papers reported that lignin content, one of the most abundant biopolymers resistant to decomposition, was negatively correlated with decay rate, especially in the cases of root and wood plant tissues included in the sample of analysed litters (Fogel and Cromack, 1977; Meentemeyer, 1978). Consistent negative correlations with litter decay rates have been also reported for carbon-to-nitrogen content (C/N) (Taylor et al., 1989) and

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0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.03.003

lignin-to-nitrogen content (lignin/N) ratios (Melillo et al., 1982). In detail, Taylor et al. (1989) reported that C/N, for substrates with limited lignin content (e.g. understory herbs) predicted litter decay rate better than lignin/N. In any case, both C/N and lignin/N ratios have been extensively used in most C-cycle models, such as CENTURY (Parton et al., 1994), BIOME-BGC (Hunt et al., 1996) and Linkages (Pastor and Post, 1986), as descriptors of litter quality controlling mass loss rate (Burke et al., 2003; Adair et al., 2008).

However, litter quality continuously changes during decomposition being function of the relative susceptibility to breakdown of its organic components, with rapid degradation of labile simple sugars, and selective preservation of less degradable tannins and lignin (Rovira and Vallejo, 2007). Berg and Matzner (1997) developed a three-phase model with labile organic compounds and nutrients (i.e. N and P) controlling decay rate up to 30–40% of mass loss, and lignin content becoming progressively more important afterwards. The model has been successfully tested on a range of litter types including hardwoods and coniferous plants (Berg and McClaugherty, 2008). Further modelling attempts to take into account progressive chemical changes during decomposition have been implemented (Liski et al., 2005; Adair et al., 2008; Incerti et al., 2011), with organic matter quality described by litter mass partitioning into different pools, exponentially decaying at different rates according to first-order kinetics (Olson, 1963).

In spite of research efforts and of positive evidences, both parameters and indexes currently used to describe litter quality and changes during decomposition are not free of uncertainty. For instance, Berg and McClaugherty (2008) suggested that using C/N ratio to predict leaf decay rate throughout the decomposition process should be avoided, because, irrespectively of its initial value, C/N progressively decreases as C is lost by respiration and N is immobilized in microbial biomass (Bonanomi et al., 2010). Recently, Goebel et al. (2011) reported that decomposition of fine roots of four temperate tree species were opposite to what might been expected from C/N ratio. Moreover, Hättenschwiler et al. (2011) reported that both C/N and lignin/N ratios poorly predict litter decomposition rate of many tree species of lowland Amazonian forest.

In the last decade, chemical throughput methods as pyrolysis-gas chromatography/mass spectrometry (Huang et al., 1998), near infrared reflectance spectroscopy (Gillon et al., 1999) and ^{13}C-cross-polarization magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Kögel-Knabner, 2002) have been applied to characterize organic matter at molecular level. In particular, ^{13}C-CPMAS NMR has been proven useful to provide a description of the total organic chemical composition of complex matrices, such as plant litter (Kögel-Knabner, 2002), allowing to obtain the resonance signals of all the carbons of the analyzed samples. Since the chemical shifts of different C atoms depend on their molecular environment, important information about their chemical type and the nature and number of substituents allows the attribution of observed carbons to a particular class of organic compounds. Then, by analysing litter samples at different decomposition stage, the changes of different classes of organic C corresponding to different levels of litter decay can be assessed. Several studies based on ^{13}C-CPMAS NMR data reported a continuous chemical shift during litter decomposition, with a progressive decrease of carbohydrates and increase of lipids and lignin (Preston et al., 2009; Bonanomi et al., 2011). As a consequence, some indexes of organic matter stability have been proposed, such as the ratios hydrophobic to hydrophilic C (HB/HI) and alkyl to O-alkyl C (alkyl/O-alkyl), both consistently increasing during decomposition (Almendros et al., 2000; Preston et al., 2009; Ono et al., 2011), and the ratio carbohydrate to methoxyl C (CC/MC), steeply dropping down at the initial stages of the decay process (Mathers et al., 2007; Bonanomi et al., 2011). More recently, Bonanomi et al. (2011) proposed a new index of organic matter stability based on ^{13}C-CPMAS NMR that was highly correlated with litter phytotoxicity. Such index, calculated as the direct ratio between two restricted regions of NMR spectra (i.e. 70–75/52–57) included major contribution of C2, C3, and C5 signals of carbohydrates (70–75 ppm) as well as methoxyl C signal of lignin (52–57 ppm).

These evidences, in contrast to traditional C/N and lignin/N indexes, provided a promising possibility of using ^{13}C-CPMAS NMR data to operationally define litter quality but, so far, no systematic comparison between these different approaches has been reported. Instead, this has been the general objective of this study. To this purpose, sixteen different plant litter types, decomposing in microcosm under optimal controlled conditions to avoid limiting effects of water availability and temperature on litter decay rates (Taylor et al., 1989; Gholz et al., 2000), were analysed in a litter-bag experiment. A previous report, based on the same litter decomposition experiment, showed the possibility of monitoring by ^{13}C-CPMAS NMR the quality changes of organic C during the decomposition process thus allowing the prediction of the litter inhibitory effects on plant growth (Bonanomi et al., 2011). Specific objectives of this second contribution were:

(1) to test the relationship between litter decay rate and the C/N and lignin/N ratios from the decomposing materials at different decay stages;
(2) to analyse by ^{13}C-CPMAS NMR the organic C dynamics related to litter decomposition;
(3) to assess the possibility of using empirical indices and statistical models based on ^{13}C-CPMAS NMR data to predict litter decomposition rate;
(4) to compare ^{13}C-CPMAS NMR data and C/N and lignin/N ratios, as functional indicators of litter quality and predictors of litter decay rates.

2. Materials and methods

The current work is based on a previous litterbag decomposition experiment focused on phytotoxic effects of plant litter (Bonanomi et al., 2011).

2.1. Plant material collection

A pool of plant species representing a wide range of litter quality were selected from vegetation types of Mediterranean and temperate environments (Campania Region, Southern Italy) including two grasses (*Ampelodesmos mauritanicus*, *Festuca drymeia*), one forb (*Medicago sativa*), two evergreen shrubs (*Arbutus unedo*, *Coronilla emerus*), one vine (*Hedera helix*), three evergreen trees (*Picea excelsa*, *Pinus halepensis, Quercus ilex*), and seven deciduous trees (*Castanea sativa*, *Fagus sylvatica*, *Fraxinus ornus*, *Populus nigra*, *Quercus pubescens, Robinia pseudoacacia*, *Salix alba*). Three species are nitrogen-fixing (*M. sativa*, *C. emerus*, *R. pseudoacacia*). For each species, a number >20 of individuals from natural communities was randomly selected at the sampling sites, and freshly abscised leaves were collected by placing nets under the plants, dried (40 °C until constant weight was reached) and then stored at room temperature.

2.2. Litter decomposition experiment

Decomposition experiments were carried out according to the litterbag method (Berg and McClaugherty, 2008) in microcosms placed in a growth chamber to simulate field decomposition