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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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 using 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 $^{75/52}$ 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 $^{70}$ ratio is proposed as an alternative to C/N and lignin/N ratios for application in experimental and modelling work.

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1. Introduction

Leaf litter decomposition is a critical process for biogeochemical cycling in terrestrial ecosystems (Attwill 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...
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 (Parson 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 Valdejo, 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 conifers plants (Berg and McClaugherly, 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 McClaugherly (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 be 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(380,662),(651,727) 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 microcosms 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 absceded 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 McClaugherly, 2008) in microcosms placed in a growth chamber to simulate field decomposition
conditions. Microcosms were kept under controlled temperature (18 ± 2 °C night and 25 ± 2 °C day) and water (watered every seven days to field capacity with distilled water). Terylene litterbags (20 × 20 cm, mesh size 2 mm) were filled with 6 g of dry leaf litter and placed inside trays (30 cm deep, 100 cm for each side). A total of 384 litterbags (16 species × 3 sampling dates × 8 replicates) were harvested after 30, 90 and 180 days of decomposition. Bags were dried at the laboratory (40 °C until constant weight was reached) and the remaining material weighted afterwards (for details see Bonanomi et al., 2011).

2.3. Litter chemical analyses and 13C-CPMAS NMR characterization

Total C and N content in litters from the litterbag experiment were determined by flash combustion of microsamples (5 mg of litter) in an Elemental Analyser NA 1500 (Carlo Erba Strumentazione, Milan, Italy). The content of acid-detergent hydrolysable fibre (thereafter indicated as labile C), proximate cellulose and lignin were assessed following the procedure described by Gessner (2005). Briefly, labile C was determined by mild acid hydrolysis with 0.5 M H2SO4 added with the detergent cetyltrimethylammonium (CTAB, 20 g l−1). Proximate cellulose was determined as hydrolysable fraction after drastic sulphuric acid treatment (loss due to 72% H2SO4 for 3 h), and proximate lignin as the unhydrolysable fraction (loss upon ignition after the above mentioned H2SO4 treatment). It is important to note that the lignin assessed in this way does not correspond to pure lignin because it may include some other hydrolysis-resistant organic structures, such as cutin, waxes, and condensed tannins at varying proportions (Berg and McClaugherty, 2008; Preston et al., 2009). All carbon fractions are presented as ash-free dry mass.

All litters types were characterized by 13C-CPMAS NMR in solid state under the same condition, thus allowing a quantitative comparison among NMR spectra. Litter samples were analysed at four different times, corresponding to leaves decomposition for 0, 30, 90 and 180 days (total of 64 litter types). A spectrometer (Bruker AV-300, Bruker Instrumental Inc, Billerica, MA, USA) equipped with a magic angle spinning (MAS) probe with wide-bore of 4 mm was used, set up with MAS of 13,000 Hz of rotor spin, a recycle time of 1 s, a contact time of 1 ms, an acquisition time of 20 ms, and 2000 scans (for details see Bonanomi et al., 2011).

Spectral regions have been selected and C types identified as reported in Bonanomi et al. (2011), following previous reference studies (Kögel-Knabner, 2002; Mathers et al., 2007; Pane et al., 2011). The following seven regions and C types were considered: 0–45 p.p.m = alkyl C; 46–60 p.p.m = methoxyl C; 61–90 p.p.m = O-alkyl C; 91–110 p.p.m = di-O-alkyl C; 111–140 p.p.m = H- and C-substituted aromatic C; 141–160 p.p.m O-substituted aromatic C (phenolic and O-ary C); 161–190 ppm carboxyl C. Four indexes, which are considered robust indicators of the degree of litter decomposition, were calculated: i. the hydrophobicity index (HB/Hl); ii. the alkyl C/O-alkyl C ratio (0–45/61–110), and; iii. the O-alkyl C/methoxyl and N-alkyl C ratio (61–90/46–60; thereafter indicated as CC/NC) (Spaccini et al., 2000; Almendros et al., 2000; Kögel-Knabner, 2002) and described above. For all GLMs, significant differences between groups were tested using Tukey’s HSD post hoc test.

A previous comparative analysis of existing decomposition equations (Rovira and Rovira, 2010), showed that different decomposition datasets can be best fitted by different equations. Testing of simple–exponential equation (Olson, 1963), double-exponential (Gillon et al., 1994), single-compartment with variable rate (Yang and Janssen, 2000), and composite-exponential (Rovira and Rovira, 2010) models were performed on our datasets. Results are shown in supplementary material (Table S1). Considering that the sample size was not adequate for the application of best fitting models, requiring a higher number of parameters, we applied litter negative exponential decay constant (k) calculated according to Berg and McClaugherty (2008) by applying the Olson (1963) model. The model equation was Mt = M0 e−kt, where M0 is the initial litter mass, Mt is the mass remaining after a certain time t, and k is the decay rate constant. Three consecutive exponential models have been applied to three different decomposition periods (0–30, 30–90 and 90–180 days). Both univariate and multivariate statistics were used to address the relationship between litter decay rate (k) and litter chemical parameters (i.e. N, labile, cellulose and lignin content, C/N ratio, lignin/N ratio, data from 13C-CPMAS NMR spectra). Simple correlation was extensively tested between litter decay rate and each parameter, including all 13C-CPMAS NMR spectral signals. Using a multivariate approach, principal component regression (PCR) was performed. Such analysis consists of a multiple linear regression (MR) in which principal component analysis (PCA) is used to calculate independent variables (Jolliffe, 2002) to avoid multicollinearity (i.e. a significant correlation between two or more predictor variables in a multi-regressive model). A multi-regressive model was tested, in which the seven spectral regions were considered as predictive variables for litter decay rate. Based on a matrix of litter types and spectral regions, independent linear combinations of the predictors were calculated by PCA. The correlation between litter decay rate (k) and each principal component was calculated using k values and the factorial scores of litter samples. Following the approach suggested by Legendre and Legendre (1998) for supplementary variables, litter decay rate was also plotted as a loading vector on the bi-dimensional PCA space even if it was not used to compute the eigenvalues of the same ordination space. Finally, the four orthogonal PCA axes most correlated with litter inhibitory effects were used as independent predictive variables in the multiple regression model. The model was fitted using data from 30 litter samples obtained by randomly selecting 10 plant species and 3 decomposition stages from the complete dataset. Thereafter, the model was validated on litter samples of the remaining 6 plant species, by comparing predicted vs observed litter decay rate.

3. Results

3.1. Dynamics of litter decomposition

Litter mass loss largely varied among different plant species, and for each species over time (Fig. 1A), with decay rates (k) ranging between 18.8 and 0.31. Mass loss was initially extremely rapid for C. emerus, H. helix, and F. ornus, and slower for Q. ilex and P. excelsa, showing intermediate values for other species (Fig. 1A). However, as decomposition proceeded, mass loss declined for all litter types, with rates decreasing over time during the decay process (Fig. 1A).
Percent content of N and lignin in decaying materials progressively increased during the 180 days of decomposition (Fig. 1B). Both parameters were significantly different among litter types (Table 1). Highest initial N content was found in nitrogen-fixers C. emerus (3.53%) and M. sativa (3.94%), and lowest in A. mauritanicus (1.19%) and Q. ilex (1.34%). Percent lignin content ranged from values slightly above 5% of undecomposed herb and vine materials (C. emerus and H. helix) to values over 40% of litters from trees after 180 days of decomposition, with maximum (50.13%) observed for Q. ilex. However, variations over time were statistically significant only in the case of lignin, but not for N concentration (Table 1).

Undecomposed litters showed very different values of C/N ratio, ranging between 40 (A. mauritanicus) and 11 (M. sativa) with mean ± standard error of 22.9 ± 7.0. High interspecific variability was also found in the case of lignin/N ratio, with mean ± standard error of 9.5 ± 5.1. As decomposition proceeded, the C/N and lignin/N ratios showed contrasting dynamics, with a decrease and a slight increase, respectively (Fig. 1C). Both these trends were significantly affected by litter type and decomposition time (Table 1). A significant interactive effect of litter type and decomposition time was found for the ratio lignin/N (Table 1) indicating that the increase of such parameter with decomposition time was significantly different among the considered litter types, being more pronounced in materials with high lignin/N ratio, and less evident in litters with lower lignin/N.

13C-CPMAS NMR spectra showed remarkable and consistent changes of organic C components in litters, with all spectral regions significantly affected by litter type (Table 1), and major chemical shifts observed for the initial decomposition period (Fig. 2). The alkyl-C region (0–45 ppm), characteristic of lipid waxes and cutins, significantly increased during the first 30 days of decomposition (Fig. 2), and much less later on. The methoxyl and N-alkyl C region (46–60 ppm) showed a progressive increase, with significant differences between initial, intermediate, and final values (Fig. 2). The O-alkyl-C region (61–90 ppm), mainly associated with sugars and polysaccharides, steeply decreased at the initial decomposition stage, then showing a continuous but not significant reduction (Fig. 2). The di-O-alkyl-C region (91–100 ppm) showed a similar pattern, with significant reduction during the first 30 days of decomposition, but with a less pronounced reduction (Fig. 2). Other regions, including H- and C-substituted aromatic C, O-substituted aromatic C, and carboxyl C, though significantly affected by litter type (Table 1), were not affected by significant changes during decomposition (Fig. 2, Table 1). Both the reduction of the O-alkyl-C and the increase of the alkyl-C regions were more evident for fast decomposing materials (e.g. leaf litter of H. helix, C. emerus, F. orrus) than for slow decaying ones (e.g. P. excelsa, Q. pubescens, F. sylvatica). The degree of hydrophobicity (HB/HI) and the ratio alkyl-C/O-alkyl-C showed a significant increase during decomposition. Finally, the CC/MC and the 70–75/52–57 ratios rapidly decreased in the first 30 days and then showed a further progressive reduction (see Bonanomi et al., 2011).

### 3.2. Relationships between litter decay rate and traditional indices of litter quality

The ratios C/N and lignin/N from undecomposed materials showed negative correlations with litter decay rate in the early decomposition stage (0–30 days, Fig. 3). However, different results were obtained considering C/N and lignin/N from already decomposing materials, which showed non-significant correlation with decay rates both at intermediate (30–90 days) and late (90–180 days) decomposition stages (Fig. 3). Interestingly, in these latter cases, Pearson’s r values were almost null for lignin/N, and even shifted towards positive values in the case of C/N (Fig. 3).

Concerning other elemental and proximate chemical parameters, different patterns were observed. Labile C and N content showed significant positive correlations with decay rate only at the early decomposition stage (0–30 d). Lignin and cellulose content showed the opposite trend, with significant and non-significant negative correlation, respectively, at the early decomposition stage (0–30 d; see supplementary material, Table S2). However, none of the considered parameters was significantly related, either positively or negatively, with decay rate of already decomposing materials (Table S2).

### 3.3. Predictivity of the 13C NMR-based PCR model of litter decay rate

Principal component analysis (PCA) provided a satisfactory ordination of the 13C-CPMAS NMR data across litter types (Fig. 4), with the first four eigenvalues accounting for 98.5% (54.9%, 24.9%,...
11.5%, and 7.2%) of the total variance, respectively. The multiple regression analysis based on the results of PCA, aimed at assessing the relationship between $^{13}$C-CPMAS NMR data and litter decay rate, provided a highly significant linear model (Table 2). The model, once applied to the validation dataset, provided very highly fitting predictions of litter decay rates (predicted vs observed values: $r = 0.91$) (Fig. 4).

### 3.4. Relationship between litter decay rate and dynamics of selected organic C types

Litter decay rate was not significantly related with three $^{13}$C-CPMAS NMR regions (Carboxyl C, O-substituted aromatic C, and H- and C-substituted aromatic C, with $r = 0.08$, $-0.07$, and $-0.19$, respectively). A significant negative correlation was found in two cases, including alkyl C ($r = -0.38, P < 0.01$) and methoxyl and N-alkyl C regions ($r = -0.46, P < 0.001$), whereas a significant positive correlation was found for the O-alkyl C ($r = 0.48, P < 0.001$) and di-O-alkyl C ($r = 0.37, P < 0.01$) regions. Concerning indices derived from the literature (Spaccini et al., 2000; Almendros et al., 2000; Kögel-Knabner, 2002; Bonanomi et al., 2011) litter decay rate was negatively correlated with the Alkyl C/O-alkyl C ($r = -0.35, P < 0.05$) and the hydrophobic/hydrophilic (HB/HI, $r = -0.46, P < 0.001$) ratios, and positively with the carbohydrate C/methoxyl C (CC/MC) ratio ($r = 0.51, P < 0.001$). An extensive correlation analysis of all signals of the $^{13}$C-CPMAS NMR spectra from litter types showed several restricted regions significantly related to litter decay rate. The strongest positive and negative coefficients were found for two restricted fractions of the O-alkyl C (70–75 ppm) and the methoxyl and N-alkyl C (52–57 ppm) regions, respectively, with higher absolute values compared to the corresponding wide regions. A simple empirical index calculated as the ratio between the relative abundance of the two restricted regions (i.e. 70–75/52–57; Bonanomi et al., 2011) showed the most consistent correlation pattern with litter decay rate among all the parameters tested in this study (Fig. 5). This was evident considering spectral data from undecomposed materials and short-, middle-, and long-term decay
rates, as well as already decomposing materials, at different decomposition stages (Fig. 5). In fact, for all datasets, the correlation was positive and significant.

4. Discussion

C/N and lignin/N ratios are known as predictors of litter decay rate. In this work we found that this is true only in the case of undecomposed materials. In fact, such indices were not significantly associated to litter decay rates when applied to already decomposed materials, both showing contrasting correlation with mass loss in the different phases of the decomposition process. On the other hand, the proposed approach based on $^{13}$C-CPMAS NMR analysis provided consistent improvement of litter decay rate predictions, by the complex, but highly fitting multivariate model based on PCR. Interestingly, the simple empirical index $70 \text{e}^{75}/52 \text{e}^{57}$, differently from the traditional indices, maintained a consistent positive correlation with litter decay rate during all decomposition phases, thus providing a simpler straightforward application of NMR spectral data.

Our findings can be explained as follows: the progressive reduction of litter decay rate during decomposition indicates a progressive deterioration of litter biochemical quality likely related to: (i) the fast decay of labile C fraction, progressively reducing the relative content of more easily degradable matter; (ii) the progressive decrease of available nutrients; (iii) the slow decomposition of resistant C fractions, with consequent accumulation of persistent organic compounds (e.g. lignin). In this context, C/N and lignin/N ratios seem unable to describe all the observed changes of litter quality, with limited predictivity of decomposition dynamics related to changes of N concentration and C quality observed during the decay process. The increase of N concentration during decomposition of the 16 plant species litter is consistent with previous findings from a range of environments and litter types (Berg and McClaugherty, 2008). However, it is also known that N content has a dual, contrasting effect on litter decomposition, enhancing the decay rate during the early stages (up to 30–40% of mass loss) and limiting it thereafter (Berg and Matzner, 1997). At the beginning of the process, high N content sustains large microbial populations that rapidly consume labile C compounds, resulting in a high mass loss rate. In contrast, high N concentration at the later stages inhibits mass loss by favouring the formation of recalcitrant chemical complexes with lignin, though the microbiological and biochemical mechanisms have not yet fully clarified (Hatakka, 2001). In fact, Berg et al. (1996) reported a higher decomposition limit (i.e. the amount of mass remaining at which the decay rate approaches zero) in nitrogen-rich litters with initially fast decay, compared to more lignified plant tissues, decomposing at an initially lower, but less variable rate. The observed pattern of correlation between litter N content and mass loss rate, with opposite signs at different phases of decomposition, is consistent with the foregoing rationale, and partially explains the reasons why C/N ratio fails in describing litter quality during the whole decomposition process. Besides, C/N ratio has a further,

![Fig. 3. Correlation (Pearson's r coefficient and associated P-value) between decay rate (k) and C/N (left) and lignin/N (right) ratios of 16 plant species litters. Top to bottom figures refer to early, intermediate, and late decomposition stages.](image-url)
inclusion in datasets used for either of decomposition, based on factorial scores. Litters are symbolized according to these Legendre (1998). Middle: trajectories of plant species litters between 0 and 180 days the regression model (see also Table 2). For each predictive factor in the models, estimates for regression coefficient (β: raw value; β*: standardized), associated standard errors (SEs), and statistical significance are reported. The model equation is: \( k = -0.608 * \text{PC I} + 1.005 * \text{PC II} + 0.220 * \text{PC III} - 0.261 * \text{PC IV} + 3.103 \).

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* The β coefficients are obtained by first standardizing all the variables, thus allowing comparison of the relative contribution of each independent variable in the prediction of the dependent variable.

intrinsic limit: this index, by including the amount of total organic C, does not take into account the biochemical dynamics affecting the C quality during the decomposition process. By analogy with C/N, also lignin/N ratio, showing negative correlation with litter decay rate at early stages of decomposition, and either positive or not significant thereafter, likely undergoes the contrasting effects of N on litter decay rate. Consistently, the simple lignin content, devoid of information on N, showed higher correlation with litter decay rate compared to lignin/N ratio. Interestingly, labile C and N content, which are auto-correlated, showed similar relationship with decay rate with positive correlation at early stages, but not significant association at later decomposition stages. Again, these results could be due to the contrasting effects of nitrogen, or eventually to the accumulation of C compounds identified as labile by proximate analyses, but yet resistant to decomposition. This inconsistency may reflect the limitations of proximate analytical methods, which cluster, as mentioned before, a range of different compounds (Gessner, 2005). The amount of these compounds often increases during the decomposition process (Berg and McClaugherty, 2008). For instance, in a long-term study, Preston et al. (2009) found that, during decomposition, lignin alone could not account for the observed increase of acid unhydrolyzable fractions. Other studies will investigate further the characterization of different litter types by pyrolysis-gas chromatography/mass spectrometry analysis (Huang et al., 1998) and differential thermogravimetry calorimetry (Rovira et al., 2008).

\(^{13}\)C-CPMAS NMR has been used to assess the suppressive capability of organic matter to soilborne pathogens (Pane et al., 2011), and to describe litter biochemical quality (Preston et al., 2009; Ono et al., 2011) with the purpose of predicting litter mass loss (Almendros et al., 2000). Our statistical model based on

**Fig. 4.** Principal component regression (PCR) between \(^{13}\)C-CPMAS NMR spectral regions and decay rate (k) of litter types. Top: loading vectors of spectral regions. Litter decay rate (k) is also plotted as a supplementary variable following Legendre and Legendre (1998). Middle: trajectories of plant species litters between 0 and 180 days of decomposition, based on factorial scores. Litters are symbolized according to these inclusion in datasets used for either fitting (open circles) or validation (filled circles) of the regression model (see also Table 2). Bottom: predicted and observed decay rates for litters of the validation dataset.

**Fig. 5.** Correlation (Pearson’s r coefficient and associated P-value) between litter decay rate (k) and the empirical index \( 70-75 / 52-57 \), based on restricted fractions of the O-alkyl C (61–90 ppm) and methoxyl and N-alkyl C (46–60 ppm) \(^{13}\)C NMR spectral regions. The linear equation was: \( k = 1.55 \times (70-75 / 52-57) - 3.10 \).
5. Conclusions

Our study provided clear-cut evidence that the use of C/N and lignin/N ratios as descriptors of litter quality is limited to unde-composed materials, with the same indices unable to predict mass loss of already decomposed litter. In contrast, an approach based on $^{13}$C-CPMAS NMR analysis led to more predictive descriptions of organic matter quality throughout the whole investigated decomposition process. We showed that the relationship between litter quality and its decay rate can be satisfactorily predicted by multivariate statistical models based on $^{13}$C-CPMAS NMR spectral regions. Besides, a new index of litter biochemical quality ($70–75/52–57$) provided a consistent improved correlation with litter decay rate, compared to both proximate chemical analyses and other $^{13}$C-CPMAS NMR indexes from literature. We are aware that our experiment was based on a not exhaustive number of litter types, decomposing under optimal conditions of temperature and water content. Consequently, further studies should investigate the consistency of the proposed indexes under more limiting conditions, extending the analysis to materials from other ecosystems (e.g. agro-ecosystems, grasslands, boreal and tropical forests). A practical limit of our study might be related to the limited availability of solid state $^{13}$C NMR spectroscopy, which is, so far, accessible to few laboratories. However, we showed that this method provides good insights on the chemical dynamics of decomposition processes. Further comparative studies between $^{13}$C NMR spectroscopy and other analytical methods will eventually support the identification of other indicators for litter mass predictions.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2012.03.003.

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