

Changes in chemical composition of litter during decomposition: a review of published ^{13}C NMR spectra

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Abstract

The aim of the study was to evaluate changes in chemical composition of litter during early stages of decomposition based on a comparative analysis of published ^{13}C NMR spectra. We collected over 130 ^{13}C NMR spectra from peer-reviewed articles that contained spectra for undecomposed litter and for litter in at least one stage of decomposition. We measured the areas of peaks representing individual chemical components and interpreted the proportions of those peak areas as proportions of respective chemical components. Results indicate that fresh herb litter tends to contain more carbohydrates and less phenolic compounds and to have lower remaining mass, indicating faster decomposition. Carbohydrates consistently decrease during decomposition, while the proportions of aromatic and aliphatic compounds increase. Changes in the proportions of individual chemical components are negatively correlated with their initial content. The proportion of aromatic components correlates positively with the C/N ratio and negatively with the decomposition constant.

Keywords: ^{13}C CPMAS NMR, litter decomposition, litter quality, soil organic matter

1. Introduction

Soil is a major part of the global carbon cycle (Schlesinger, 1990). Litter is considered to be a key organic source of carbon input into the soil, so litter decomposition plays a crucial role in the Earth's carbon budget. Litter decomposition influences soil biogeochemistry of plant stands and affects the formation of soil organic matter (SOM). Due to its decomposability litter affects

organic carbon accumulation in the soil and, consequently, stabilization of SOM (Lv and Liang, 2012). Differences across ecosystems are the result of different climates, varying litter input into the soil (Aranda and Comino, 2014), and changing availability of litter carbon for decomposition and potential carbon stabilization in soil (Six *et al.*, 2002). SOM, in turn, determines

important soil properties, including its fertility. The mechanisms of carbon stabilization in soils are still not well understood, although they have received much attention recently (Lützow *et al.*, 2006). The stabilization of SOM entails physical protection and chemical changes. Physical protection of soil organic matter comprises binding of SOM into soil aggregates and coating by clay minerals (Oades, 1984). Chemical changes include the formation of humic substances, which are either aromatic (humic and fulvic acids) or aliphatic (humins) in character (Aiken, 1985; Song *et al.*, 2011; Abakumov *et al.*, 2013). There is still some uncertainty, however, which compounds in litter have greater relative stability in the soil and which compounds contribute most to the formation and stabilization of SOM (Lorenz *et al.*, 2007).

Litter quality is considered to be one of the most important drivers of litter decomposition (Kara *et al.*, 2014). Especially critical for organic matter dynamics are the C/N ratio and the content of nitrogen and lignin (Swift *et al.*, 1979; Bonanomi *et al.*, 2013).

Litter quality is widely studied through relationships between litter stoichiometry and the rate of decomposition (e.g. Hobbie and Vitousek, 2000). By contrast, much less attention has been paid to chemical compositional changes of organic carbon in litter during decomposition processes in the soil. The technique of solid state ^{13}C NMR spectroscopy allows the non destructive collection of data about individual components of litter material. This powerful method is useful for observing the chemical behaviour of litter material and evaluating the quality of organic matter. Small sample amounts provide structural information about the material studied without the need to extract it. We focused our research on aliphatic, aromatic and carbohydrate components. The objective of this study is to evaluate the patterns of changes in chemical composition of litter during the process of decomposition. We especially focused on aliphatic and

aromatic compounds, which have been considered as the fundamental components of stable organic matter.

2. Methods

Using the keywords “litter decomposition”, “litter decay” and “ ^{13}C NMR spectroscopy”, we searched the Web of Science (<http://apps.webofknowledge.com>) for papers which published original ^{13}C NMR spectra. There are more related, especially early, NMR literature which was not possible to include in this study (e.g. Hempfling *et al.*, 1987; Preston *et al.*, 2000; Baldock *et al.*, 1992). The articles either do not contain useable NMR spectra or approach litter decomposition in different way. For that reason it would not be possible subsequent evaluation of those data. Overall, it was extracted over 130 NMR spectra covering almost fifty various cases of decaying litter from twelve different articles (Table 1). These span various types of litter decomposing under various climatic conditions. For each study, at least two spectra, representing initial plant material and less decomposed (<1y) litter, were available. For many of the studies, spectra representing more decomposed litter (>1y) were also available. These spectra were used to quantify the basic carbon fractions according to Wilson (1987) based on the area of corresponding peaks relative to the total area of all peaks in the spectra using WebPlotDigitizer software (<http://arohatgi.info/WebPlotDigitizer>). The work is focused on chemical shift regions representative of three types of carbon components: aliphatic (10–45 ppm), carbohydrate (60–90 O-alkyl and 90–110 acetal and ketal groups) and aromatic (110–160 ppm). The content of individual chemical components of litter was quantified for each plant species. For the purpose of this study, the plant species were divided into several groups: conifers, broadleaf trees, herbs and evergreen shrubs.

Table 1. Peer-reviewed journals used as sources of NMR spectra for undecomposed litter and litter in at least one stage of decomposition

Reference	Collected NMR spectra (n)	Reference	Collected NMR spectra (n)
Almendros <i>et al.</i> 2000	24	Mathers <i>et al.</i> 2007	16
Carvalho <i>et al.</i> 2009	15	Ono <i>et al.</i> 2009	8
De Marco <i>et al.</i> 2012	10	Ono <i>et al.</i> 2011	8
Frouz unpublished data	3	Ono <i>et al.</i> 2013	14
Lorenz <i>et al.</i> 2000	6	Quideau <i>et al.</i> 2005	9
Lorenz <i>et al.</i> 2004	8	Webster <i>et al.</i> 2000	10

In addition, the C/N ratio and information about climate conditions given in the articles were used for purpose of this study. The decomposition constant (k value) was calculated from remaining mass data available in the articles. Missing climate data were replaced (mean annual temperature, mean annual precipitation) by values obtained from www.weatherbase.com using geographic coordinates or described locations. Climatic conditions were categorized as follows: tropical, Mediterranean-subtropical, temperate and boreo-alpine.

Because there are no replications for the individual spectra, a classical meta-analysis was not performed, but individual NMR spectra were treated as single data points. Each spectrum was therefore considered as an individual statistical entry. The shifts of individual carbon fractions were compared between initial litter and decomposed litter using a pair t test. The difference was expressed as the percentage in decomposed litter minus the percentage in original litter. This was calculated for all available entries as well as for entries divided according to the duration

of decomposition (less than one year and more than one year) and according to the type of plant (conifer, broadleaf tree, herb, evergreen shrub). Using a one-way ANOVA, differences in the proportions of the same chemical categories among various types of litter were tested. Linear correlation coefficients were calculated between initial percentages of the given chemical components and the shifts in corresponding group percentages during decomposition. Similarly, correlation coefficients were calculated between initial percentages of the given chemical categories and the C/N ratio of the litter, and between the same percentages and the decomposition constant k . All the mentioned calculations were carried out by Statistica 10.0.

Principal component analysis (PCA) was performed by Canoco 4.0 to visualize the variation in the proportions of individual chemical components (based on NMR spectra) among litter types considered in individual studies in relation to the type of plant, climatic zone, litter C/N ratio and decomposition constant.

3. Results

3.1. Initial litter quality

The ^{13}C NMR litter spectra show that carbohydrates constitute the largest proportion in initial litter (Table 2). This proportion, expressed as percentages of the total peak area of carbon in O-alkyl and acetal and ketal groups, ranges from 33.18 and 10.70 to 44.92 and 11.06 %, respectively, depending on the litter type group. Fresh litter also contains a large proportion of aliphatic components (16.43 to 25.96 %). Aromatic components are less variable in fresh litter (11.37 to 17.52 %). The relative content of carbon types expressed as the percentage of the total spectral area is shown in Table 2. Despite certain tendencies apparent in some litter types, there is no significant difference in the chemical composition of fresh litter among the litter types under consideration except for aromatic compounds from herbs. Litter of evergreen shrubs and conifers tends to be higher in recalcitrant (aliphatic and aromatic) compounds. Herb litter is characterized by higher content of easily degradable compounds (carbohydrates). With regard to the climate, boreo-alpine litter contain significantly more aliphatic compounds. Litter in

temperate areas tends to contain higher amounts of aromatic compounds, and Mediterranean-subtropical litter is characterized by higher amounts of carbohydrates. The C/N ratio positively correlates with the content of aromatic components (see Table 3). Temperate litter has a significantly higher C/N ratio than tropical litter (Figure 1). Evergreen and boreo-alpine litter has higher C/N ratios, too. These tendencies are also apparent in the PCA ordination diagram. Its first ordination axis is related to the gradient between carbohydrates and aliphatic components whereas the second component is related to the presence of aromatic compounds. Herbs ($p < 0.05$) correlate with the first ordination axis (0.285). It is characterized by correlation with the content of carbohydrates. The second ordination axis correlates with differences between temperate and tropical plants, which significantly positively or negatively correlate with the second axis (0.348 and -0.361). The second axis also positively correlates with the C/N ratio. The initial C/N ratio is negatively correlated with the decomposition constant k (-0.3547). Initial content of aromatic components is negatively correlated with k . The decomposition constant is positively correlated with initial concentrations of carbohydrates, but there is no correlation between k and initial aromaticity/aliphaticity ratios.

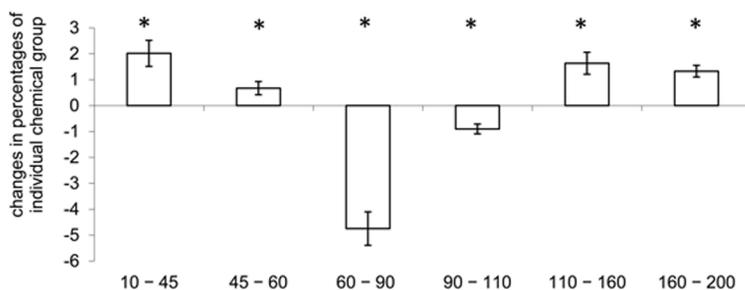


Figure 2. Changes in percentages of individual chemical components between the initial and the final stage of litter decomposition. Values are means \pm SEM. Changes marked by asterisks are statistically significant

Table 2. Percentages of individual chemical components (means± SEM) in individual types of litter. Statistically homogeneous groups of litter types in the same stages of decomposition are marked by the same letters (ANOVA, LSD post hoc test $p < 0.05$). Asterisks mark significant differences between initial conditions and decomposed litter based on a pair t test (initial vs decomposed litter, $p < 0.05$). If decomposed litter was collected repeatedly, several pairs were tested, so the n in each t-test corresponds to the n for the given decomposed litter

Corresponding chemical component	Chemical shift region [ppm]					n	
	10–45	45–60	60–90	90–110	110–160		160–200
	Aliphatic		Carbohydrates		Aromatic		
<i>Initial</i>							
Conifer	21.36 ±1.35	7.74 ±0.43	38.43 ±1.69	10.90 ±0.50	16.16 ±0.56a	5.42 ±0.53a	12
Broadleaf tree	19.38 ±0.93	8.34 ±0.33	39.02 ±1.18	11.26 ±0.38	15.55 ±0.40a	6.45 ±0.41a	22
Herb	16.43 ±1.28	7.45 ±0.53	44.92 ±1.78	11.06 ±0.60	11.37 ±0.66b	8.75 ±0.66b	9
Evergreen shrub	25.96 ±1.85	5.19 ±0.84	33.18 ±2.53	10.70 ±0.89	17.52 ±0.70a	7.45 ±1.05a	4
<i>Less than one year</i>							
Conifer	21.85 ±1.36	8.85 ±0.62ab	33.51 ±1.89	10.40 ±0.57	18.82 ±0.92a	6.57 ±0.43	6
Broadleaf tree	23.26 ±1.20*	9.35 ±0.30b	32.21 ±1.39*	9.79 ±0.44*	17.47 ±0.62ab	7.93 ±0.53*	15
Herb	17.31 ±1.07	7.20 ±0.73a	40.41 ±6.41*	10.64 ±1.00	15.11 ±5.13b*	9.33 ±2.54	22
Evergreen shrub	27.03 ±3.39	7.99 ±0.92ab	30.89 ±1.91	11.54 ±0.98	17.32 ±2.27a	5.23 ±0.60	3
<i>More than one year</i>							
Conifer	21.93 ±0.92	8.99 ±0.39	33.17 ±1.23*	10.38 ±0.39	19.02 ±0.59*	6.51 ±0.29*	14
Broadleaf tree	23.81 ±1.58*	9.03 ±0.33*	34.52 ±1.29*	9.54 ±0.40*	14.79 ±0.56	8.31 ±0.44*	14
Herb	14.92 ±1.67	6.76 ±0.47	35.25 ±2.46	10.91 ±0.43	20.53 ±1.04	11.64 ±0.93	4
Evergreen shrub	25.05 ±0.52	7.49 ±0.02*	25.89 ±0.25*	9.98 ±0.24	19.72 ±0.28	11.87 ±0.81	2

The patterns of changes in chemical composition during decomposition vary the most in the early stage of litter decomposition. Decomposition patterns in the late stage are highly similar among litter types.

We found changes in individual chemical components to be negatively correlated with the original content of the same components. Substances that are more abundant in initial litter tend to decrease more during decomposition (Table 3).

Table 3. Correlation coefficients between the initial percentages of given functional groups and shifts in the percentages of those groups during decomposition. Separate computations were made for periods shorter and longer than one year and for both of these periods together. The two bottom rows contain correlation coefficients between the initial percentage of the given functional group and the C/N ratio and k , respectively. Only significant r values are presented

	Chemical shift regions [ppm]					
	10–45	45–60	60–90	90–110	110–160	160–200
Corresponding chemical component	Aliphatic		Carbohydrate		Aromatic	
Early <1y		-0.79242				
Late >1y	-0.48828	-0.67056	-0.44315	-0.62977	-0.7259	-0.35184
Both	-0.17173	-0.7305		-0.45493	-0.46738	
Correlation with C/N					0.419237	
Correlation with k			0.36481		-0.41919	

The C/N ratio decreases during litter decomposition. The decrease depends more on the value of the C/N ratio at the start of the decomposition (0.605, $p < 0.01$) than on the decomposition period (0.262, non significant).

4. Discussion

The collected data demonstrate a significant increase in the proportion of both aliphatic and aromatic compounds during litter decomposition, although the proportions do not necessarily increase in all cases. Aromatic compounds have been widely assumed to be highly resistant to decomposition and have therefore been thought to control litter decomposition (De Marco *et al.*, 2012), which is in agreement with our observation. A signal indicating aromatic

substance comes mainly from lignin and tannins. Aromatic compounds have been regarded as the main contributors to the formation of humic substances. This is supported also by classical theory that aromatic components form substantial portion of humic substances: fulvic and humic acids. On the other hand, the accumulation of aromatic compounds is not necessarily the dominant process taking place during humification (Lorenz *et al.*, 2000; Ono *et al.*, 2000), and aromatic compounds may not necessarily be the building blocks of humic substances (Almendros *et al.*, 2000). Data from a recent study (Abakumov *et al.*, 2013) show that decomposition of litter with a higher content of lignin and a higher lignin/N ratio leads to a higher proportion of low-molecular fulvic acids and that litter with a lower lignin/N ratio leads to humic acids with higher aromaticity.

Our results also show increase in aliphatic alkyl structures that accumulate during litter decomposition which is in agreement with other studies (Baldock *et al.*, 1992; Almendros *et al.*, 2000). Most studies have revealed a relative increase in alkyl intensity over the duration of the decomposition process (Lorenz *et al.*, 2000; Lorenz *et al.*, 2004; Quideau *et al.*, 2005; Carvalho *et al.*, 2009; De Marco *et al.*, 2012; Bonanomi *et al.*, 2013). The alkyl intensity peak is assumed to come mainly from recalcitrant surface waxes, cutin, suberin, lipids and amino acids. The increase of aliphatic carbon compounds in litter of different types could also be caused by microbial synthesis (Hopkins *et al.*, 1997). Aliphatic carbon compounds could increase due to cross-linking of long-chain alkyl compounds during humification (Kögel-Knabner *et al.*, 1992). Recently, several studies (e.g. Almendros *et al.*, 2000; Mathers *et al.*, 2007) have emphasized the role of aliphatic compounds as a resistant fraction of litter, which is in agreement with our results. Our results also corroborate the recent observation that humin, the most recalcitrant organic soil fraction, is composed mainly of aliphatic hydrocarbons (Simpson *et al.*, 2002; Song *et al.*, 2011), and hence relative accumulation of the alkyl fraction during decomposition may play an important role in the stabilization of soil organic matter. Our observation (Figure 2) that aliphatic components of litter increase during decomposition supports this idea. We therefore assume that the proportion of aliphatic compounds in litter could play a part in stabilizing soil organic matter including aromatic components of litter.

The collected data clearly demonstrate a progressive decrease in carbohydrate-derived carbon. This trend is obvious in all of the studies. According to Almendros *et al.* (2000), carbohydrates do not systematically decrease in all species. However, our results show a decrease of carbohydrates in

all groups considered. Carbohydrates are easily degradable compounds. Their decay, however, can sometimes be slowed down by the ligninocellulose complex. This happens because saccharides bind to lignin and thus evade decomposition.

The negative correlation between the initial C/N ratio and decomposition constant k is in agreement with most studies (Bonanomi *et al.*, 2013); a low initial C/N ratio of litter indicates subsequent higher loss of plant biomass during the decomposition, and conversely, a higher initial C/N ratio indicates slower decay of litter.

Litter decomposition constants differ from one species to another. Litter properties affect decomposition constants differently among litter types (litter from conifers, broadleaf trees, herbs and evergreen shrubs) and among climatic categories of litter (tropical, Mediterranean-subtropical, temperate and boreo-alpine). In general, mass loss diminishes gradually along a gradient from herb litter, through broadleaf tree and coniferous litter, to evergreen shrub litter (Preston *et al.*, 2000). Our data also support this pattern and show that it is mostly caused by chemical composition. Even climatic groups are characterized largely by their chemical composition. For example, Mediterranean shrubs are often characterized by a thick waxy cuticle, which is important in decomposition (Quideau *et al.*, 2005). In our study, we found a strong gradient in litter quality between temperate and tropical plants. This finding has to be taken with caution because the plants whose litter was considered in the available studies may not be representative of the vegetation in the given regions. On the other hand, our results, which show that temperate plants are more associated with higher C/N ratios and higher lignin content, are in agreement with the findings of Aerts (1997) that temperate plants have significantly higher C/N ratios than tropical ones.

5. Conclusion

As already mentioned in the introduction, both stoichiometry and chemical composition of organic matter play important roles in litter decomposition. Our results support this; there is a significant correlation between the decomposition constant k and initial content of carbohydrates and aromatic compounds. Our results, however, also show that there is a strong correlation between the C/N ratio and initial content of aromatic components, which means that stoichiometry and organic matter composition are at least partly linked.

There is an obvious increase in the content of aromatic and aliphatic compounds during litter decomposition. We therefore expect these components to play relevant roles in humification processes and, consequently, SOM stabilization.

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