Effect of lignin concentration on CO_2 emissions in forest soils of the Sierra Nevada

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ABSTRACT

Objective: To evaluate the effect of lignin concentration in leaf litter on the mineralization rate and cumulative mineralization in forest soils.

Design/methodology/approach: Twenty-five grams of soil from an *Abies religiosa* forest in the Sierra Nevada were incubated with increasing concentrations of lignin from leaf litter and branches, at 60% humidity and 35 °C. CO_2 was captured in a 0.5 N NaOH and 0.5 N barium chloride solution, and titrated with 0.5 N H_2SO_4. A completely randomized experimental design with two factors was used. Mineralization rate and cumulative mineralization were determined. Linear regression analysis and ANOVA were performed using the SAS OnDemand for Academics statistical package.

Results: CO_2 emissions followed a linear model for both lignin and soil levels, with mineralization rates ranging from 12.06 mg CO_2 day^{-1} to 33.68 mg CO_2 day^{-1}. There were highly significant differences between lignin concentrations. The interaction between plot and lignin concentration also showed statistically significant differences. A positive and highly significant relationship was found between soil nitrogen and phosphorus content.

Limitations on study/implications: It is recommended to consider climate as a year-round source of variation in CO_2 emissions, as well as its interaction with leaf litter quality and microbial activity.

Findings/conclusions: CO_2 emissions exhibit a linear and positive trend with increasing lignin concentration. Both mineralization rates and cumulative mineralization show statistically significant differences across lignin levels and total nitrogen content, as well as in the interaction between plot and lignin concentration.

Keywords: Organic soil carbon, recalcitrant carbon, C:N, humus.

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INTRODUCTION

Mineralization is the biological transformation of organic compounds into simple inorganic forms. The mineralization of soil organic carbon is the main cause of carbon dioxide (CO_2) emissions into the atmosphere. This process is influenced by temperature, moisture, soil characteristics, microbial diversity and structure, enzymatic activity, and type of vegetation. Temperature is one of the most influential factors in mineralization,

as increases or fluctuations in temperature affect the mineralization rate (Huang *et al.*, 2019) (1).

CO_2 release into the atmosphere in forest systems originates mainly from the mineralization of organic carbon compounds contained in leaf litter, such as sugars, proteins, phenols, hydrocarbons, and glycerides. The proportion of these carbon forms varies across plant structures and species, including cellulose, hemicellulose, and lignin, which are components of the cell wall. Lignin accounts for 15% to 40% of the total leaf litter although in some cases it may range from 4% to 50%. It is an extremely flexible molecule with a variable structure (Krishna & Mohan, 2017) (2). This biopolymer, composed of phenolic heteropolymers, is the most abundant and recalcitrant form of carbon on Earth, resistant to microbial degradation, and the main source of humus. Lignin contributes to the stabilization and cycling of soil organic carbon (Wang *et al.*, 2019) (3). In addition, lignin plays an important role in the biogeochemical cycles of other elements, as well as in maintaining soil fertility. In this context, lignin and cellulose are the main organic components involved in the mineralization of leaf litter (Li *et al.*, 2016) (4). The amount and composition of lignin released into forest soils from organic residues are influenced by environmental conditions such as temperature, moisture, solar radiation, and the mineralization rate (Stutz *et al.*, 2019) (5). Lignin is a complex organic molecule that represents approximately 20% of plant residue composition. It is resistant to enzymatic degradation, meaning that the lignin content in plant tissue is negatively correlated with its decomposition. Its content, composition, and structure vary among plant species. The quality of this plant material influences its decomposition dynamics and, consequently, the stabilization of organic matter in the soil. Decomposition models for plant residues suggest that recalcitrant carbon compounds with high lignin content decompose more slowly than residues rich in soluble sugars. The biochemical decomposition of recalcitrant carbon forms is more sensitive to temperature fluctuations compared to labile carbon forms. Moreover, the enzymatic kinetics involved in the decomposition of recalcitrant compounds increase at higher temperatures, as greater energy is required to activate the associated biochemical processes (Stewart *et al.*, 2015) (6). Among the variables correlated with litter mineralization, the carbon-to-nitrogen (C:N) ratio, the lignin-to-nitrogen (L:N) ratio, and cellulose content stand out. These factors have been used as predictors of organic residue decomposition (Talbot & Treseder, 2012) (7). The C:N ratio is one component among a set of variables that significantly influence the mineralization of organic residues in the soil. However, there is a linear relationship between mineralizable nitrogen and lignin concentration, making the lignin content in organic materials an indicator of residue mineralization when applied to soil (Hernández-Mendoza *et al.*, 2007) (8). This ratio has been used as an indicator of nitrogen levels in the surface layers of forest soils and of mineralization; however, it is influenced by the lignin and nitrogen content of leaf litter and other residues from different plant species. Species with high lignin content and low nitrogen decompose more slowly (Cools *et al.*, 2014) (9). This biochemical process is limited by the lability of carbon and the availability of nitrogen (Fujii *et al.*, 2020) (10).

Forest soils are the main terrestrial reservoir of organic carbon (40%); however, their storage capacity is finite, as the mineralization of organic carbon is estimated to occur after the maximum accumulation of organic carbon in the form of humus in the organic horizon of mineral soils (Prescott & Vesterdal, 2021) (11). Based on the above, the objective of this research was to estimate the effect of lignin concentration on the mineralization rate and accumulated mineralization of organic carbon in forest soils.

MATERIALS AND METHODS

Soil samples were collected at a depth of 0 to 10 cm in the oyamel fir forest (*Abies religiosa*), located at 19° 25.609' N latitude and 98° 45.785' W longitude, on Mount Tláloc in the Sierra Nevada, eastern State of Mexico. The altitude ranges from 3,093 to 3,489 meters above sea level. The climate is semi-cold, with an annual average temperature between 5 °C and 12 °C. Annual precipitation ranges from 800 to 1,200 mm. pH, Soil Organic Carbon (SOC), Organic Carbon (OC), Soil Organic Matter (SOM), Cation Exchange Capacity (CEC) were determined (Table 1).

Soil incubation was carried out in airtight 200 ml jars. Twenty-five grams of soil (air-dried in the shade and sieved to 2 mm) were placed in each jar, adjusted to 60% of its water holding capacity, and incubated at 35 °C. The leaf litter incorporated into the soil consisted of a mixture of needles and branches, ground and sieved through a 40-mesh sieve. To determine lignin concentrations, a weighted average was used, based on neutral detergent fiber and acid detergent fiber analysis values of the leaf litter samples (Table 2). The amount of leaf litter mixture incorporated into the soil was estimated based on the annual leaf litterfall at each sampling plot (P1: 3.0 t ha^{-1} $year^{-1}$; P2: 3.9 t ha^{-1} $year^{-1}$; P3: 2.5 t ha^{-1} $year^{-1}$; P4: 2.6 t ha^{-1} $year^{-1}$; and P5: 2.5 t ha^{-1} $year^{-1}$).

CO_2 emissions were captured using a 0.5 N NaOH solution. In each jar, a test tube containing 5 mL of the NaOH solution was placed and replaced at each measurement. CO_2 readings were taken daily during the first four days, every two days for the

Table 1. Characteristics of the soils used for determining the carbon mineralization rate in forest soils.

Plot	Altitude (m)	pH	SOC	OC	SOM	CEC meq 100 g^{-1} S	N	P
			%				mg kg^{-1} S	
P1	3489	5.8	4.2	7.4	12.8	34.8	18.8	6.2
P2	3360	6.1	3.0	4.6	7.8	37.3	19.5	6.4
P3	3359	6.4	2.0	6.5	11.2	38.0	21.2	13.0
P4	3359	6.2	2.5	4.4	7.6	37.5	23.4	21.8
P5	3093	6.2	2.3	4.0	6.9	36.7	30.5	17.4

SOC: Soil Organic Carbon; OC: Organic Carbon; SOM: Soil Organic Matter; CEC: Cation Exchange Capacity; N: Nitrogen; P: Phosphorus.

Table 2. Characteristics of needles and branches of *Abies religiosa* used in the incubation.

Litterfall	Litterfall dry matter (g)	Ashes	Cellulose	Hemicellulose	Lignin	Total nitrogen	L:N
		%					
Needles	97.3	5.1	21.8	14.5	11.6	1.2	9.8
Branches	96.1	4.4	28.9	13.1	21.9	0.8	28.2

L: Lignin; N: Nitrogen.

following eight days, then every three days for six days, and finally on day 28 after the start of the incubation. For the quantification of the recovered CO_2, the NaOH solution was mixed in an Erlenmeyer flask with 2.5 mL of 0.5 N barium chloride solution to precipitate the adsorbed CO_2. Two drops of phenolphthalein were added, and the solution was titrated with 0.5 N H_2SO_4. A completely randomized experimental design with two factors (plots and lignin concentrations) and three replicates was used. The mineralization rate and cumulative mineralization were determined from the data. These variables were analyzed using linear regression, analysis of variance (ANOVA) and multiple mean comparison (Tukey). The relationships between variables were evaluated through linear correlation (Pearson). The statistical package SAS OnDemand for Academics was used.

RESULTS AND DISCUSSION

Based on the analysis of variance of the linear regression of lignin concentrations and incubation time, it was determined that CO_2 emissions fit a linear model with an alpha of 0.01 (Table 3) for all lignin levels and each soil evaluated, indicating that lignin mineralization under these conditions is independent of soil characteristics.

Table 3. Analysis of variance of the linear regression of lignin concentrations on CO_2 emissions after 28 days of incubation.

Source of variation			**Model**	**Error**	**Total**
Degrees of freedom			**1**	**40**	**41**
Sum of squares (% Lignin concentration in litterfall)	P1	0	642623**	19389	662011
		10.6	941876**	15763	957639
		13.6	830375**	89561	919936
		23.3	662203**	38007	700210
	P2	0	595126**	24731	619857
		11.8	525190**	4353.16	529543
		14.8	695654**	19051	714705
		22.2	418158**	19935	438093
	P3	0	1483852**	18077	1501930
		12	1568789**	18627	1587415
		14.5	2028942**	18150	2047091
		20.8	1485147**	45441	1530588
	P4	0	1842007**	17077	1859084
		12.1	2605616**	4929.58	2610546
		14.5	2381299**	16876	2398175
		23.1	2194099**	56817	2250917
	P5	0	2279219**	42675	2321894
		11.5	2767987**	35590	2803577
		13.6	3263165**	43630	3306796
		20.2	3125949**	63182	3189131

*Significant=0.05; **Highly significant=0.01; ns: not significant.

The linear regression analysis determined that CO_2 emissions show a linear trend between the lignin concentrations added to each soil and the CO_2 recovered in the five soils evaluated after 28 days of incubation, with mineralization rates ranging from 12.06 mg day^{-1} of CO_2 to 33.68 mg day^{-1} of CO_2 (Table 4). Similarly, the coefficient of determination ranged from 0.90 to 0.99, indicating that more than 90% of CO_2 emissions depend on the incubation time.

The linear behavior is independent of the added lignin concentration, since when comparing the CO_2 emissions from soils with 0% lignin, similar rates are observed among the applied concentrations (Figure 1). Klotzbücher *et al.* (2011) (12) mention that lignin mineralization in soil mainly depends on the inputs of labile carbon and other soluble forms that provide energy to the microbial biomass.

The analysis of variance for the effect of plot and lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization indicates that there are highly significant differences between the individual factors for both the mineralization rate and cumulative mineralization. Meanwhile, for the interaction between plot and lignin concentration, highly significant differences were found in the mineralization rate and significant differences in cumulative mineralization (Table 5).

The multiple mean comparison analysis of the plot effect on the mineralization rate indicates that the highest rate occurred at plot 5, with 31.45 mg day^{-1} of CO_2, which

Table 4. Lignin mineralization rates at 28 days of incubation.

Plot	Lignin concentration in litterfall (%)	Lignin mineralization rate (mg day^{-1})	r^2
1	0	14.95	0.97
	10.6	18.09	0.98
	13.6	16.99	0.90
	23.3	15.17	0.95
2	0	14.38	0.96
	11.8	13.51	0.99
	14.8	15.55	0.97
	22.2	12.06	0.95
3	0	22.71	0.99
	12	23.35	0.99
	14.5	26.56	0.99
	20.8	22.72	0.97
4	0	25.30	0.99
	12.1	30.10	1.00
	14.5	28.77	0.99
	23.1	27.62	0.97
5	0	28.15	0.98
	11.5	31.02	0.99
	13.6	33.68	0.99
	20.2	32.96	0.98

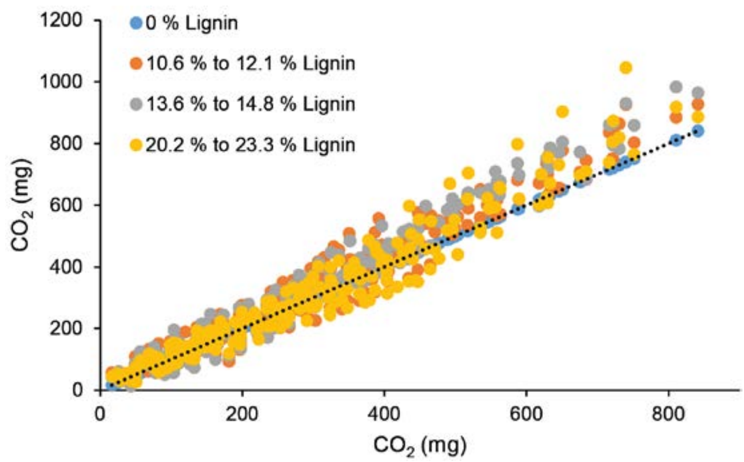

Figure 1. Relationship between CO_2 emissions and lignin concentration in the soil.

Table 5. Analysis of variance for the effect of plot and lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization.

SV	DF	Lignin mineralization rate ($mg\ day^{-1}$)		Cumulative mineralization (g)	
		SS	F-value	SS	F-value
Plot	4	2690.80	286.13**	67.80	173.46**
LC	3	86.57	12.27**	2.24	7.63**
Plot-LC	12	75.98	2.69**	3.82	3.25*
Error	40	94.04		3.91	
Total	59	2947.40		77.76	
R^2		0.97		0.95	
CV		6.76		7.11	

SV: Source of variation; DF: Degrees of freedom; SS: Sum of squares; LC: Lignin concentration in litterfall; CV: Coefficient of Variation; *Significant=0.05; **Highly significant=0.01; ns: not significant.

corresponds to a total nitrogen content of 30.5 mg kg^{-1} S. In contrast, at plot 2, the mineralization rate was 13.88 mg day^{-1} of CO_2, with a nitrogen content of 19.5 mg kg^{-1} soil (Table 6). These CO_2 emissions suggest a relationship between nitrogen content and the mineralization rate, showing that lower soil nitrogen content is associated with lower mineralization rates.

Regarding cumulative mineralization, statistical differences were observed among the plots, with P5 showing the highest CO_2 accumulation and P2 the lowest, with 5.98 g and 2.95 g, respectively. Based on these data, and considering that soil nitrogen content plays a role in the mineralization process through the C:N ratio, both the rate and cumulative mineralization are influenced by the nitrogen content in the soil. This, in turn, determines the activity of microorganisms involved in mineralization, as residues with a low C:N ratio and high N content undergo faster mineralization. This is because microorganisms use nitrogen for cell growth and reproduction. It has been estimated that when the C:N ratio exceeds 25, nitrogen in the soil tends to become immobilized (Pei *et al.*, 2019) (13).

Table 6. Multiple mean comparison of the plot effect on the rate of organic carbon mineralization and cumulative organic carbon mineralization.

Plot	Lignin mineralization rate (mg day^{-1})	Cumulative mineralization (g)
P5	31.45 a	5.98 a
P4	27.95 b	5.04 b
P3	23.84 c	4.41 c
P1	16.30 d	3.59 d
P2	13.88 e	2.95 e
MSD	1.79	0.36

MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

The addition of lignin to the soil showed statistically significant differences in both the mineralization rate and cumulative mineralization, compared to soils without lignin addition. The highest mineralization rate and cumulative mineralization were observed with the addition of 13.6% to 14.8% lignin (Table 7). Based on the CO_2 emissions determined in this experiment, a linear pattern was observed across all simulations. However, the statistical differences found in the mineralization rates indicate an influence of the amount of lignin present in the soil on its resistance to microbial mineralization. This is because carbon quality affects both the composition of microbial communities and the total microbial biomass. For example, in the presence of recalcitrant forms of carbon, such as lignin, fungi tend to dominate (Ali *et al.*, 2018) (14).

The resistance of lignin to mineralization is due to the fact that it is a form of carbon that provides little energy to microorganisms, as it is protected by cellulose compounds. Microorganisms expend more energy breaking down this structure than the energy they gain from its mineralization (Rinkes *et al.*, 2016) (15).

The multiple comparison analysis indicates that the interaction Plots-Lignin Concentration shows statistically significant differences between the mineralization rate

Table 7. Multiple comparison of means for the effect of lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization.

Lignin concentration in litterfall (%)	Lignin mineralization rate (mg day^{-1})	Cumulative mineralization (g)
LC3	24.31 a	4.63 a
LC2	23.22 ab	4.50 a
LC4	22.11 bc	4.34 ab
LC1	21.10 c	4.11 b
MSD	1.5007	305.96

LC1: 0% Lignin; LC2: 10.6%-12.1% Lignin; LC3: 13.6%-14.8% Lignin; LC4: 20.2%-23.3% Lignin; MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

and the accumulated mineralization (Table 8). The highest mineralization rates ranged between 33.68 and 30.10 mg CO_2 day^{-1} in the interactions P5-LC3, P5-LC4, P5-LC2, and P4-LC2, which correspond to the plots with the highest nitrogen content of 23.4 mg kg^{-1} soil and 30.5 mg kg^{-1} soil, respectively. This suggests that nitrogen content has a greater influence on the mineralization rate than lignin concentration. This condition is similar for accumulated mineralization.

The correlation analysis (Table 9) suggests a positive and highly significant relationship between total soil nitrogen, extractable phosphorus, and both the mineralization rate and cumulative mineralization, with coefficients of 0.83, 0.87; 0.86, and 0.79, respectively. This is because high levels of soil nitrogen stimulate microbial respiration (Ma *et al.*, 2020) (16). Furthermore, the mobilization and mineralization of organic compounds of C, N, and P are highly correlated; that is, immobilization and mineralization have a strong relationship with microbial activity (Brödlin *et al.*, 2019) (17).

Table 8. Multiple comparison of means for the effect of the plot and lignin concentration interaction on the rate of organic carbon mineralization and accumulated organic carbon mineralization.

Plot-LC	Lignin mineralization rate (mg day^{-1})	Plot-LC	Cumulative mineralization (g)
P5-LC3	33.68 a	P5-LC3	6.37 a
P5-LC4	32.96 ab	P5-LC4	6.32 a
P5-LC2	31.02 abc	P5-LC2	5.90 ab
P4-LC2	30.10 abc	P5-LC1	5.33 bc
P4-LC3	28.77 bcd	P4-LC2	5.31 bc
P5-LC1	28.15 cd	P4-LC3	5.13 bc
P4-LC4	27.62 cde	P4-LC4	4.97 bcd
P3-LC3	26.56 cdef	P3-LC3	4.87 cd
P4-LC1	25.30 def	P4-LC1	4.76 cd
P3-LC2	23.35 ef	P3-LC2	4.46 cde
P3-LC4	22.72 fg	P3-LC4	4.15 def
P3-LC1	22.71 fg	P3-LC1	4.14 def
P1-LC2	18.09 gh	P1-LC2	4.07 def
P1-LC3	16.99 h	P1-LC3	3.79 efg
P2-LC3	15.55 hi	P1-LC4	3.49 fgh
P1-LC4	15.17 hi	P2-LC1	3.31 fgh
P1-LC1	14.95 hi	P1-LC1	3.02 gh
P2-LC1	14.38 hi	P2-LC3	2.98 gh
P2-LC2	13.51 hi	P2-LC4	2.77 h
P2-LC4	12.06 i	P2-LC2	2.76 h
MSD	4.7427	MSD	0.9669

LC1: 0% Lignin; LC2: 10.6%-12.1% Lignin; LC3: 13.6%-14.8% Lignin; LC4: 20.2 %-23.3% Lignin; MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

Table 9. Correlation of soil characteristics with litter mineralization.

Correlation	pH	SOC	OC	SOM	CEC (meq 100 g^{-1} S)	N	P
		%					
Lignin mineralization rate (mg day^{-1})	0.53	−0.65	−0.47	−0.46	0.32	0.83**	0.87**
Cumulative mineralization (g)	0.42	−0.55	−0.44	−0.42	0.19	0.86**	0.79**

SOC: Soil Organic Carbon; OC: Organic Carbon; SOM: Soil Organic Matter; CEC: Cation Exchange Capacity; N: Nitrogen; P: Phosphorus; *Significant $\alpha=0.05$; **Highly significant $\alpha=0.01$; ns: not significant.

CONCLUSIONS

CO_2 emissions show a positive linear trend in relation to the concentration of added lignin. Mineralization rates and cumulative mineralization exhibit statistically significant differences due to the addition of increasing levels of lignin to the soil. Plots with higher total nitrogen content present higher mineralization rates and cumulative mineralization compared to plots with lower total nitrogen content. Significant differences exist in mineralization rate and cumulative mineralization in the interaction between plots and lignin concentration.

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