Mineralization of Carbon and Nitrogen from Freeze- and Oven-Dried Plant Material Added to Soil

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ABSTRACT

Drying organic material before soil incorporation is a common procedure used in mineralization or decomposition studies. A laboratory study was conducted to determine the effect of drying methods on plant C and N and associated mineralization patterns in soil. Freeze- and oven-dried water hyacinth (Eichhornia crassipes [Mart] Solms) was added to a Kendrick soil (loamy, siliceous, hyperthermic Arenic Paleudults) at a rate of 5 g kg⁻¹ and incubated in the dark at 27 °C for 90 d. Oven drying in paper bags significantly increased the lignin content and decreased the mineral content of the plant material compared to freeze drying. The total C and N was not significantly different for the two materials. The mineralization of C from freeze-dried plant material was more rapid during the initial stage of decomposition and remained significantly higher throughout the incubation. At 90 d, 50 and 41% of the plant C had evolved as CO₂ for the freeze- and oven-dried plant material, respectively. Mineralization of ¹⁵N from the plant material accounted for 33% of the applied N of the oven-dried material and 23% of the applied N of the oven-dried material. Nitrogen mineralization and CO₂ evolution were linearly correlated (r = 0.998) for the oven-dried plant material, but less correlated (r = 0.770) for the freeze-dried material.

Drying organic material before soil incorporation is a standard procedure in mineralization or decomposition studies. Oven drying is still a common practice (Gilmour et al., 1985), but freeze drying (Jenkinson, 1965; Miller, 1974) and air drying (Herman et al., 1977) have been used. Dalal (1979) oven dried plant material at 60 °C in a forced draft oven to minimize changes in C and P compounds as compared to air drying. Miller (1974) assumed that freeze drying sludge would not chemically alter the organic compounds or influence the rate at which the microbial population would decompose the sludge after soil incorporation. A comparison of the effect of drying techniques on distribution of plant C and related mineralization patterns in soil has not been made.

Van Soest (1965) reported erroneous measurements of lignin when forages were oven-dried. A procedure for measuring cell wall constituents (Goering and Van Soest, 1970) stated that heat drying of forages at temperatures above 50 °C produced analytically significant increases in yield of lignin. Goering and Van Soest (1970) attributed the increase of lignin to the production of artifact lignin via a nonenzymatic browning reaction that involved plant N. This reaction required water and involved the condensation of carbohydrate degradation products with protein or amino acids to form an insoluble polymer (Donoso et al., 1962). If oven drying does convert a portion of the soluble C or N to a more decomposition-resistant form, then oven drying of organic material may lead to erroneous conclusions about subsequent mineralization in soil.

The objective of this laboratory study was to compare mineralization of C and N of freeze- and oven-dried water hyacinth added to soil, as measured by CO₂ evolution and N₂O-N accumulation. Water hyacinths have been used extensively to treat wastewater due to their potential productivity in nutrient enriched waters (Wolverton and McDonald, 1979; Reddy et al., 1985). The plant material has been evaluated as an organic soil amendment (Parra and Hortonstine, 1976) and as feedstock for anaerobic digestion (Shiralipour and Smith, 1984). There is considerable interest in the decomposition characteristics of this plant.

MATERIALS AND METHODS

Water hyacinths were collected from a wastewater retention pond of the Reedy Creek Utility Company at Walt Disney World near Orlando, FL. The hyacinths were grown in a nutrient solution containing ¹⁵N labeled (NH₄)SO₄ for 2 wk to obtain ¹⁵N labeled plant material. The concentrations of added nutrients were: ¹⁵NH₄-N = 20.0 mg L⁻¹; K = 23.5 mg L⁻¹; PO₄-P = 3.1 mg L⁻¹; Ca = 20.0 mg L⁻¹; Mg = 4.6 mg L⁻¹; SO₄-S = 6.4 mg L⁻¹; and Fe = 6.0 mg L⁻¹.

Micronutrients were applied through a commercially available liquid fertilizer (Nutrispray-Sunniland, Chase and Co., Sanford, FL) to obtain an initial concentration of 0.2 mg Cu L⁻¹, 1.5 mg Mn L⁻¹, 0.04 mg B L⁻¹, and 0.02 mg Mo L⁻¹.

The plants were frozen at -10 °C for 1 wk to facilitate chopping to a 1.6-mm length by passing the material through a Hobart T 215 food processor. This length was chosen for subsequent studies on anaerobic digestion of water hyacinth (K. K. Moorhead and R. A. Nordstedt, 1986, unpublished results, Univ. of Florida). The effects of freezing the plant material on plant C and N were not determined.

A portion of the chopped water hyacinths was freeze-dried (Thermovac T) and another portion was oven-dried in a forced-air convection oven at 70 °C for 1 wk. Both sets of dried materials were ground to pass through the 0.84-mm screen of a Wiley mill. The freeze-dried and oven-dried plant materials were characterized for volatile solids and ash, total C (LECO Induction Furnace 523-300, LECO Corp., St. Joseph, MI), lignin, cellulose, and hemicellulose (Goering and Van Soest, 1970), total Kjeldahl nitrogen (TKN) (Nelson and Sommers, 1973), and ashed mineral constituents (Gaines and Mitchell, 1979). Volatile solids and ash were calculated from loss on ignition at 500 °C in a muffle furnace.

Surface (0- to 15-cm depth) soil samples of a Kendrick fine sand were collected from a fallow field at the Agronomy Farm, University of Florida, Gainesville. The soil was air-dried and passed through a 2-mm sieve. The soil had a particle-size distribution of 929 g sand kg⁻¹ soil, 46 g silt kg⁻¹, and 25 g clay kg⁻¹. The cation exchange capacity (CEC) was 3.44 cmol, kg⁻¹ soil, with a base saturation of 47%. The initial pH was 5.44 in water (1:2, w/v) and the organic C and TKN contents were 5.8 g and 0.38 g kg⁻¹ soil, respectively.

Fifty-gram soil samples were preincubated for 5 d at a water content equivalent to 10 kPa suction before addition of the plant materials. The preincubation was to equilibrate the soil to a constant water content. The freeze- and oven-dried plant materials were thoroughly mixed with the soil at a rate of 5 g (dry wt.) kg⁻¹ soil (equivalent to 10 Mg ha⁻¹). They were incubated in the dark for 90 d at 27 °C. Each treatment consisted of three replications.
The water content of the soil samples was adjusted every 15 d by adding deionized water gravimetrically to a soil moisture equivalent of 10 kPa suction. Ambient air, with CO₂ and NH₃ removed by 3 M NaOH and 4 M H₂SO₄ traps, respectively, was pumped through the incubation flasks with an aquarium pump at a rate of 50 mL min⁻¹. The CO₂ evolved from the soil samples was collected in traps containing 50 mL of 0.1 M NaOH. The trapped CO₂ was determined by titration with standard acid after precipitation of carbonate with BaCl₂. The percentage C evolved with time was calculated by subtracting C evolved as CO₂ from the control soil (no organic C amendment) from the two treatments and dividing by the amount of C added.

Soil samples were analyzed at 0, 30, 60, and 90 d for 2 M KCl-extractable NH₄-N and NO₃-N by steam distillation (Keeney and Nelson, 1982) and for TKN by acid digestion (Nelson and Sommers, 1972). The ¹⁵N analyses of plant and soil samples were conducted on a Micro Mass 602E mass spectrometer (VG Instruments, West Sussex, England). The results were statistically analyzed with the t statistic, using the two plant materials as treatments and dividing by the amount of C added.

Results and Discussion

Plant Material Characterization

There was no significant difference in total C content between the two plant materials, although the lignin content was significantly higher for the oven-dried plant material (Table 1). The increase in lignin content was apparently due to the production of artifact lignin when the plant material was oven-dried (Van Soest, 1965; Goering and Van Soest, 1970). Goering and Van Soest (1970) concluded that artifact lignin was produced when plant material was oven-dried at temperatures above 50 °C through a nonenzymatic browning reaction that involved plant C and N. The drying techniques used here did not affect the cellulose or hemicellulose fraction. Although no significant differences in TKN were found, the distribution of plant N (i.e., amino acids, amino sugars, proteins, etc.) was probably affected by the drying technique (Donoso et al., 1962).

Significant differences in volatile solids and the mineral content between the two plant materials were apparently due to water loss and absorption to the paper bag in which the oven-dried plant material was dried. Visible signs of water loss and absorption to the paper bag were noted. However, the paper bag was not analyzed to determine the loss of mineral constituents or soluble organics. The freeze-dried plant material was dried on a stainless steel tray. The loss of mineral constituents during oven drying may not be as critical for plant material with a low initial moisture content. Water hyacinth plants typically have a moisture content of 93 to 96% (Penfound and Earle, 1948). Glass or stainless steel containers should be used to dry organic material for decomposition studies.

Carbon Mineralization

The C released as CO₂ was significantly higher throughout the incubation for the freeze-dried plant material (Table 2). The greatest differences occurred within the first week of incubation. At the end of 4 d the percentage C evolved as CO₂ was 16.2% for the freeze-dried plant material compared to 9.0% for the oven-dried material. After 90 d of incubation, 50 and 41% of the added C had evolved as CO₂ for the freeze- and oven-dried plant material, respectively.

A multi-stage decomposition sequence, based on first-order kinetics, was used to describe the C mineralization patterns of the two materials (Reddy et al., 1980; Gilmour et al., 1985; Moorhead et al., 1987). Graphical representation of the stages and their respective rate constants are shown in Fig. 1. The stages represent the sequential ease of mineralization of each C-constituent, i.e., soluble sugars and starch, cellulose and hemicellulose, and lignin. The rate constants for the first stage were 0.0041 and 0.0235 d⁻¹ for freeze-
Table 3. Soil nitrate concentration and $^{15}$N mineralization from freeze- and oven-dried hyacinth plant material added to soil.

<table>
<thead>
<tr>
<th>Day</th>
<th>Freeze-dried Nitrate concentration ($\text{mg kg}^{-1}$)</th>
<th>$t$-test</th>
<th>Freeze-dried $^{15}$N mineralization (%)</th>
<th>Oven-dried Nitrate concentration ($\text{mg kg}^{-1}$)</th>
<th>$t$-test</th>
<th>Oven-dried $^{15}$N mineralization (%)</th>
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<tr>
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<td>0</td>
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<td>51.4</td>
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<td>**</td>
<td>26.1</td>
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<tr>
<td>60</td>
<td>70.3</td>
<td>**</td>
<td>24.8</td>
<td>40.8</td>
<td>**</td>
<td>11.1</td>
</tr>
<tr>
<td>90</td>
<td>68.7</td>
<td>**</td>
<td>34.3</td>
<td>48.9</td>
<td>**</td>
<td>16.4</td>
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** Differences significant at the 0.01 probability level.
† NS = not significant.

and oven-dried plant material, respectively. The rate constants for the second and third stages were similar for both materials.

The drying method influenced the C distribution of the plant material (Table 1) and determined how much C was available during each stage of mineralization. The fraction that mineralized rapidly was affected the most, since the production of artifact lignin involves the more soluble C and N components (Donoso et al., 1962; Van Soest, 1965). A more detailed study is required to quantify the conversion of soluble C and N components to artifact lignin.

Reddy et al. (1980) reported that decomposition of plant material in soil occurred in two stages. The first stage was considered a labile C fraction that decomposed rapidly and was related to the initial C/N ratio of the material. The second stage was a residual C fraction that was more resistant to decomposition. Gilmour et al. (1985) also concluded that plant materials essentially decompose in two stages. In this study, the mineralization of plant C was more accurately described in three stages.

Nitrogen Mineralization

Soil NH$_4$-N concentrations (data not shown) were < 2 mg kg$^{-1}$ throughout the incubation period. Nitrate concentrations in the soil during the incubation were significantly higher for freeze-dried material compared to oven-dried material (Table 3). At 30 d the NO$_3$-N concentration in soil with added freeze-dried material was approximately twice that with added oven-dried material. Maximum NO$_3$-N accumulation for the freeze-dried material occurred at 60 d compared to increasing accumulation throughout the incubation for soil with added oven-dried material. Although maximum NO$_3$-N accumulation occurred at 60 d for the freeze-dried material, C recovered as CO$_2$ continued to increase throughout the incubation.

Mineralization of organic $^{15}$N to $^{15}$NO$_3$-N accounted for 33% of the applied N from the freeze-dried material, while only 23% of the applied N was recovered as $^{15}$NO$_3$-N for the oven-dried material. Oven drying the plant material may have resulted in loss of soluble N by condensation of amino groups to carbohydrates via the nonenzymatic browning reaction (Denoso et al., 1962; Van Soest, 1965). Apparently this reaction may not occur to the same extent, if at all, during freeze drying.

Gilmour et al. (1985) established a linear relationship between net N mineralization and CO$_2$ evolution for sewage sludge and four plant materials having a high total N content or low C/N ratio. They reported that plant materials differed in their relationship between net N mineralization and CO$_2$ evolution but these relationships were highly correlated ($r > 0.93$). Gilmour et al. (1985) oven-dried plant materials at 60 °C before grinding and soil incorporation. In this study, N mineralization and CO$_2$ evolution were linear correlated ($r = 0.998$) for the oven-dried plant material, but less correlated ($r = 0.770$) for the freeze-dried material. In addition to a lower correlation, the slope of the relationship between N mineralization and CO$_2$ evolution for oven-dried material was 0.90 compared to 0.52 for freeze-dried material. Therefore, approximately 1% N was mineralized for every 1% C for the oven-dried material compared to approximately 0.5% N mineralized for every 1% C mineralized for the freeze-dried material. The differences in slope or correlation cannot be attributed to the initial C/N ratio.

The results of this laboratory incubation suggest that the method of drying affects the distribution of C in organic material and the associated mineralization patterns. Nitrogen appears to be affected by drying although the distribution of plant N was not quantified. Freeze drying appears to be a more appropriate drying method to accurately describe the mineralization of C and N during decomposition.

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REFERENCES


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