Biodynamics of microbial biomass nitrogen and sulfur in organic matter amended soil

M. A. R. Howlader¹*, A. R. M. Solaiman² and M. A. H. Chowdhury³

Abstract

An incubation experiment was carried out in a laboratory at room temperature for 60 days with four organic materials to assess the biodynamics of microbial biomass nitrogen and sulfur. The organic materials were dustbin waste, poultry litter, sewage sludge and rice straw. The organic materials were added at a ratio of 2% to the soil. A basal dressing of 200 µg P and 250 µg K/g soil in the form of KH₂PO₄ was applied to each treatment, except for the control. The highest rate of CO₂-C evolution was observed in the rice straw amended soil, which was significantly higher than that of poultry litter, dustbin waste, sewage sludge treated soil and control. Rice straw contributed the highest amounts of biomass-C, -N and -S which were significantly higher than that of poultry litter, dustbin waste and sewage sludge amended soils. The soils amended with sewage sludge, dustbin waste and poultry litter showed N and S mineralization whereas soil amended with rice straw induced N and S immobilization.

Keywords: Organic materials; Biomass; Mineralization; Immobilization

Introduction

Organic matter promotes good physical, chemical and biological conditions of soil. All organic matter in soil, whether fresh or partially decomposed, is eventually processed by the soil microbial component of the soil biota. Although microbial biomass represents a small fraction of the total soil organic matter, it has relatively rapid turn over and it exerts an important influence on soil carbon and nutrient cycling, both through the oxidation of soil organic matter and a labile reservoir of nutrient elements such as carbon, nitrogen, phosphorus and sulfur. Anderson and Domsch, 1980; Jenkinson and Ladd, 1981 Soil microorganisms play a major role not only in decomposing organic matter, but also as a sink for plant nutrients. In the decomposition of organic matter by microorganisms, most of the carbon is liberated as CO₂, the evolution of this gas can, therefore, be taken as a measure of the rate and content of the decomposition. In an arable system, the average microbial pool size is 700 kg C/ha Smith and Paul, 1990 microbial

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Howlader et al. biomass-N range from 40 to 385 kg/ha (Van Veen et al., 1985; Carter and Rennie, 1982) and the biomass-S values range from 7 kg/ha in arable system (Saggar et al., 1981a; Chapman, 1987) to 23 kg/ha in some acid organic pools (Strick and Nakas, 1984). Information about the amount and nutrient composition of the soil microbial population is necessary to understand the nature of the S transformation in soil and their relationship to C and N turnover (Saggar et al., 1981b). Moreover due to intensive crop cultivation and rapid decomposition of organic matter, every year considerable amounts of organic-C, -N and -S are lost from the soils, but continuous application of chemical fertilizers endangers soil health as well as the environment. To correct this situation, the soils should be amended with a sufficient amount of organic matter to maintain good soil health through the improvement of soil fertility every year. Organic materials such as dustbin waste, poultry litter, sewage sludge and rice straw are available for manuring purpose. However, the information on decomposition patterns and their effects on N and S dynamics and the changes in the microbial biomass in soils of Bangladesh are not available. Therefore, it is important to obtain the transformation pattern of the organic materials and their effects on the N and S dynamics and the changes of microbial biomass-N and -S. With these points in mind, a set of incubation experiments were conducted in a laboratory to determine the decomposition pattern of organic materials via dustbin waste, poultry litter, sewage sludge and rice straw. The experiments were designed to estimate the amounts of biomass-C, -N and -S formation through the addition of the organic amendments and to assess the contribution of organic materials to N and S availability in soil and to find out the assimilation percentage of N and S by the soils’ microbial biomass from added organic matter.

Materials and Methods

An incubation experiment was carried out in the laboratory of the Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) in Gazipur, Bangladesh, using Grey Terrace soil classified as Inceptisols. After sieving, a portion of soil was taken for the immediate analysis of inorganic N, SO₄-S, biomass-C, biomass-N and biomass-S, while another portion was fully air-dried to determine some physico-chemical properties of the soil (Table 1) and the remaining portion was kept for pre-incubation. Soil with 40% water holding capacity was subjected to pre-incubation aerobically at room temperature for 10 days. Pre-incubation was performed in a

<table>
<thead>
<tr>
<th>Properties</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size analysis</td>
<td></td>
</tr>
<tr>
<td>Sand %</td>
<td>46.93</td>
</tr>
<tr>
<td>Silt %</td>
<td>31.74</td>
</tr>
<tr>
<td>Clay %</td>
<td>21.33</td>
</tr>
<tr>
<td>Texture</td>
<td>Loam</td>
</tr>
<tr>
<td>Maximum water holding capacity %</td>
<td>53.16</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
</tr>
<tr>
<td>CEC mmol D⁻/kg soil</td>
<td>10.53</td>
</tr>
<tr>
<td>Organic C %</td>
<td>1.05</td>
</tr>
<tr>
<td>Organic matter %</td>
<td>1.81</td>
</tr>
<tr>
<td>Total N %</td>
<td>0.09</td>
</tr>
<tr>
<td>C: N ratio</td>
<td>12: 1</td>
</tr>
<tr>
<td>Inorganic N µg/g soil</td>
<td>14.0</td>
</tr>
<tr>
<td>SO₄-S µg/g soil</td>
<td>5.91</td>
</tr>
<tr>
<td>Biomass-C µg/g soil</td>
<td>106.56</td>
</tr>
<tr>
<td>Biomass-N µg/g soil</td>
<td>12.54</td>
</tr>
<tr>
<td>Biomass-S µg/g soil</td>
<td>1.88</td>
</tr>
<tr>
<td>Biomass C: N: S</td>
<td>57: 7: 1</td>
</tr>
</tbody>
</table>

Table 1. Physico-chemical characteristics of soils and soil microbial biomass-C, -N, and -S
plastic container, which allowed the soil microbial population to stabilize, minimizing the effects of soil handling and preparation (Chowdhury et al., 1999).

**Treatments and experimental design**

There were five treatments via non-amended control soil only (CT) and soil amended with dustbin waste (DW), poultry litter (PL), sewage sludge (SS), and rice straw (RS). The chemical properties of the organic materials (OM) are shown in Table 2. Poultry litter was collected from a local poultry farm and decomposed partially with effective microorganisms for about 2 months. The partially decomposed poultry litter was dried under open sunlight followed by oven drying at 65°C for 48 hr and passed through a 210 µm mesh sieve. Sewage sludge was collected from a local area and dried under open sunlight followed by oven drying at 65°C for 48 hr and passed through a 210 µm mesh sieve. Rice straw (Oryza sativa) were dried under open sunlight followed by oven drying at 65°C for 48 hr and passed through a 210 µm mesh sieve after grinding in a vibrating mill (Heiko Susakusho No. T-200). Prepared samples were stored in dessicators before being used for incubation. The experiment was performed in a Completely Randomized Design (CRD) with three replications. Sixty gram oven dry soil was weighed in a 100 mL glass jar and amended with organic materials at a ratio of 2% to the soil. A basal dressing of 200 µg P and 250 µg K/g soil in the form of KH₂PO₄ was applied to each treatment except for the control. Two milliliters of distilled water were added to the control soil to maintain moisture content equivalent to those of the amended soils. Following amendment, glass jars were placed in 1L glass bottles, sealed, and then incubated for 60 days at room temperature where CO₂ was trapped with 20 mL of 1M NaOH solution. Microbial biomass-C, biomass-N, biomass-S, inorganic N and SO₄-S were determined after 5, 10, 20, 30 and 60 days of incubation. Microbial respiration was monitored as CO₂ evolution from soil samples after 2, 4, 6, 10, 20, 30 and 60 days of incubation.

**Table 2. Chemical properties of the organic materials (OM)**

<table>
<thead>
<tr>
<th>Name of organic materials</th>
<th>Organic C (%)</th>
<th>Total N (%)</th>
<th>Total S (%)</th>
<th>C: N ratio</th>
<th>C: S ratio</th>
<th>C: N: S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dustbin waste</td>
<td>14.81</td>
<td>1.8</td>
<td>0.43</td>
<td>8:1</td>
<td>34:1</td>
<td>34:4:1</td>
</tr>
<tr>
<td>Poultry litter</td>
<td>19.53</td>
<td>1.27</td>
<td>0.36</td>
<td>15:1</td>
<td>54:1</td>
<td>54:4:1</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>13.86</td>
<td>0.70</td>
<td>0.60</td>
<td>20:1</td>
<td>23:1</td>
<td>23:1:1</td>
</tr>
<tr>
<td>Rice straw</td>
<td>44.5</td>
<td>0.66</td>
<td>0.07</td>
<td>67:1</td>
<td>636:1</td>
<td>636:9:1</td>
</tr>
</tbody>
</table>

**Fumigation and measurement of biomass-C, -N and -S**

The chloroform fumigation-extraction (FE) method was adopted to estimate the amount of soil microbial biomass-C (Wu et al., 1990) in the soil. The fumigated and unfumigated soils were extracted with 0.5 M K₂SO₄ (Soil: K₂SO₄ solution=1:4 for 30 minutes and filtered using Whatman No.1 filter paper and stored at 0°C. A ten milliliter extract was taken in a 100 ml conical flask and 2 ml 0.4 N K₂Cr₂O₇, 10 ml conc. H₂SO₄ and 5 ml conc. H₂PO₄ were added to it. After heating (200°C) for 30 minutes in a hot plate, it was titrated with ammonium ferrous sulfate (0.05N). The amount of biomass-C (Bc) was calculated...
from the equation: \( B_c = 2.22 \cdot E_c \) where, \( E_c \) is the difference between the organic C extracted from the fumigated and unfumigated samples. Biomass-N was measured according to the method described by Brookes et al., 1985. Biomass-C and biomass-N were measured from the same extract. The amount of biomass-N \( B_n \) was calculated from the equation, \( B_n = E_n \cdot 2.22 \) where, \( E_n \) is the difference between the amount of N extracted from the fumigated and unfumigated samples. The chloroform fumigation-extraction (FE) method was adopted to estimate the amount of soil microbial biomass-S (Wu et al., 1994) in the soil. The fumigated and unfumigated soils were extracted with 10 mM CaCl\(_2\) \( \text{Soil}: \text{CaCl}_2\) solution=1:2 for 30 minutes and filtered with Whatman No.42 filter paper and stored at 0\(^\circ\)C. Biomass-S in the extract was determined turbidimetrically. Using a spectrophotometer, the intensity of the turbidity was measured at 420 nm. The biomass-S \( B_s \) was calculated as \( B_s = F_s / K_s \), where \( F_s \) is the difference between the total S extracted from the fumigated and unfumigated soil and \( K_s \) is the factor used to convert \( F_s \) to \( B_s \). The value of \( K_s \) used was 0.31 (Wu et al., 1994). Available S \( \text{SO}_4^-\text{S} \) was extracted from the soil with a 10 mM CaCl\(_2\) solution (Page et al., 1982) and S content in the extract was determined turbidimetrically. The intensity of turbidity was measured at 420 nm using spectrophotometer.

**Statistical analysis**

The computer statistical package program MSTAT-C developed statistically analyzed collected data by Russel (1986). A factorial ANOVA was made by a F variance test. The pair comparisons were performed by a Least Significant Difference (LSD) test (Gomez and Gomez, 1984).

**Results and Discussion**

**Carbon mineralization in organic matter amended soil**

Carbon mineralization was expressed as CO\(_2\)-C evolution during the decomposition of native and added organic matter in the soil. Fig. 1 shows that the rate of CO\(_2\)-C evolution in OM amended soils at any period of incubation were higher than that of the CT soil. By day 2, the highest rate of CO\(_2\)-C evolution of 120 µg/g soil/d was found in the PL treated soil, which was significantly higher than that of 111 µg/g soil/d found in the DW, 51 µg/g soil/d in the SS and 17 µg/g soil/d in the CT soil. On the other hand, the maximum rate of CO\(_2\)-C of 477 µg/g soil/d was evolved in RS amended soil by day 6 and then sharply decreased by day 10 (Fig. 1). Here, we observed that the C mineralization in all amended soils proceeded rapidly at the initial stage of incubation and then gradually decreased. We also observed that RS with higher C: N ratio (7:1) was usually considered to complete the decomposition more slowly than those OM with lower C: N ratios (8-20:1). In contrast, higher amount of CO\(_2\)-C and slower rate of decomposition in RS was probably due to its higher C: N ratio and the presence of less decomposable organic substances e.g. cellulose and lignin etc. Plant residues with higher C: N ratios are usually decomposed more slowly than those with lower C: N ratios (Parr and Papendick, 1978; Azmal et al., 1996 a, b). Similar findings were also observed in this study.
Effect of OM on biomass-C

The amounts of biomass-C were higher in the OM amended soils than in the CT soil throughout the incubation period [Fig. 2]. In the OM amended soils, the amount of biomass-C sharply increased following an increase in the rate of CO₂ evolution [Fig. 1]. In this study, the maximum biomass-C formation in the OM amended soils ranged from 306 to 666 µg/g. By day 5, the highest amount of biomass-C of 484 µg/g was formed in the PL amended soil, which was significantly higher than that obtained with the RS (413 µg/g), DW (352 µg/g), and SS (306 µg/g) amended soils. However, in the RS amended soil, the microbial biomass-C further increased up to 666 µg/g soil by day 10 and then gradually decreased. The OM with high C: N ratio like the RS (67:1) showed a higher contribution to biomass-C formation than that of the PL, DW and SS which have lower C: N ratios (8-20:1).

Fig. 1. Rates of CO₂-C evolution in organic matter amended soils during 60 days of incubation. Bars indicate LSD at P<0.05.

Fig. 2. Changes of microbial biomass carbon in organic matter amended soils during 60 days of incubation. Bars indicate LSD at P<0.05.
Effect of OM on biomass-N

The changes in microbial biomass-N in the OM amended soils followed a pattern similar to that of the biomass-C. The amount of biomass-N sharply increased in the PL, DW and SS amended soils at day 5 and in the RS amended soil, it increased up to day 10 and thereafter decreased. By day 5, the highest amount of biomass-N of 70 µg/g was formed in the RS amended soil which was significantly higher than that of the PL (45 µg/g), DW (41 µg/g) and SS (32 µg/g) amended soil. However, the microbial biomass-N in the RS amended soil further increased by 11 µg/g soil at day 10 and then gradually decreased. There was no significant difference in biomass-N between the PL, DW, SS and CT at day 5. Rice straw, having higher C: N ratio (67: 1) showed a higher contribution to biomass-N formation than that obtained with the PL, DW and SS which have lower C: N ratios (8-20: 1). The addition of the PL, DW and SS to the soil had less effect on the amount of microbial biomass-N than the RS amended soil. Since most of the organic N contained in the readily decomposable organic materials (PL, DW and SS) with a lower C: N ratios are mineralized rapidly in soils, the soil microbial population only slightly assimilates the organic N of the added organic materials.

**Fig. 3.** Changes of microbial biomass nitrogen in organic matter amended soils during 60 days of incubation. Bars indicate LSD at P<0.05

Apparent percentage of nitrogen assimilated by microbial biomass

The apparent percentages of N assimilated by the microbial biomass from the added N at the time of the maximum biomass formation were linearly correlated with the C: N ratios of the added OM ($R^2 = 0.9995$). The apparent percentages of N assimilated by the microbial biomass from the added OM were 55% for the RS, 21% for the SS, 17% for the PL and 11% for the DW. In this study, a higher percentage of N was apparently assimilated by the microbial biomass from the OM with higher C: N ratios. For example, 55% of the added rice straw N was apparently transferred to the microbial biomass. However, in a $^{15}$N tracer study, Bremer and Van Vessel (1992) and Ocio et al., (1991b) observed that maximum values of 81% and 44% of the added wheat straw $^{15}$N were incorporated into the microbial biomass, respectively. Thus the addition of organic materials resulted in an increase in the amount of microbial biomass-N. However, the changes in the pattern of microbial biomass-N were related to the N mineralization and immobilization processes of each organic material.
Effect of OM on nitrogen transformation

The amounts of inorganic N in the DW, PL and SS amended soils were higher than in the CT soil except in the RS amended soil throughout the incubation period. The amount of inorganic N in the soils amended with the DW increased rapidly from the start of the incubation, and reached the maximum value of 70 µg/g soil which was significantly higher than 59 µg/g for the PL and 46 µg/g for the SS amended soil at day 5, which then decreased [Fig. 5]. This finding indicates that the occurrence of active N mineralization and the amount of mineralized N reached a value of 19% in the DW, 22% in the PL and 30% in the SS of the added N. Inorganic N in the soils amended with rice straw sharply decreased within 5 days where the values ranged from 1.65 to 2.96 µg/g soil. This phenomenon was attributed to N immobilization. The maximum amount of immobilization of added N was 20% in the RS amended soil. This finding is concomitant with Aulakh [1989]. He found that incorporation of wheat straw wide C: N ratio, 60: 1 increased immobilization of added-N from 12 to 29% in upland soil [at 60% saturation].

Fig. 4. Nitrogen assimilated by microbial biomass from added organic matters during maximum biomass formation. ** means significant at 1% level.

Fig. 5. Nitrogen NH₄⁺-N + NO₃⁻-N released pattern from organic matter amended soils during 60 days of incubation. Bars indicate LSD at P<0.05.
Effect of OM on biomass-S

The changes in the microbial biomass-S in the OM amended soils followed a pattern similar to that of the biomass-N. The amount of biomass-S sharply increased in the PL, DW and SS amended soils at day 5, and in the RS amended soil, it increased up to day 10 and thereafter decreased Fig. 6. By day 5, the highest amount of biomass-S of 11.72 µg/g soil was formed in the PL-amended soil which was significantly higher than that of the DW 8.78 µg/g soil and SS 4.48 µg/g soil amended soils. However, in the RS amended soil, the microbial biomass-S further increased by 2.4 µg/g soil at day 10 and then gradually declined. There was no significant difference in biomass-S between the PL and RS amended soils at day 5. Rice straw, having higher C: S ratio 636: 1 showed a higher contribution to biomass-S formation than that of the PL C: S ratio, 54: 1 DW C: S ratio, 34: 1 and SS C: S ratio, 23: 1. The incorporation of the PL, DW and SS had less effect on the amount of biomass-S than the RS. Since most of the organic S contained in the readily decomposable organic materials PL, DW and SS with lower C: S ratios 23-54: 1 were mineralized rapidly in soils, soil microbial population slightly assimilated the organic S of the added organic materials. Chowdhury et al., 2000 found marked increase in microbial biomass-S induced by the decomposition of compost resulted in assimilation of 9.4, 3.5 and 4.2 µg/g soil in cattle manure, saw dust and rice husk compost treated pots, respectively by day 5.

Apparent percentage of sulfur assimilated by microbial biomass

The apparent percentages of the S assimilated by the microbial biomass from the added S at the time of maximum biomass formation were linearly correlated with the C: S ratio of the added OM R²=0.9781 Fig. 7. The apparent percentages of the S assimilation by the microbial biomass from the added OM were 70% for the rice straw, 18% for the PL, 11% for the DW and 4% for the SS Fig. 7. Wu et al., 1993 demonstrated that 33% of the S from oil seed rape and 20% of the S from barley straw added to soil were converted to microbial biomass S after 5 days at 25.
Effect of OM on sulfur transformation

The amounts of CaCl₂-extractable SO₄-S in the SS, DW and PL amended soils were higher than in the control soil except in the RS amended soil throughout the incubation period. The amount of SO₄-S in the soil amended with SS increased rapidly from the start of incubation and reached a maximum of 46 µg/g soil, which was significantly higher than that obtained with the DW (29 µg/g) and PL (16 µg/g) amended soils at day 5 and then slightly decreased [Fig. 8]. The concentration of S in the RS amended soil was lower than in the control soil. These findings indicate that the occurrence of active S mineralization and the amount of SO₄-S reached a value of 37% in the SS, 31% in the DW, 21% in the PL and 10% in the RS of the added S. The SO₄-S concentration in the soil amended with the RS decreased within day 5 and the value ranged from 1 to 2 µg/g soil. This phenomenon was attributed to S immobilization. Turnover of S through mineralization-immobilization followed somewhat the same pattern as that of N, as both the processes occurred simultaneously. It has long been known that the C: S ratio of organic residues provide a rough guide to the amount of SO₄-S that accumulates during decay. When the C: S ratio of added plant residues is below 200, there is a net gain of SO₄-S; when the C: S ratio exceed 400, there is a net loss. For C: S ratios between 200 and 400, there is neither a gain nor loss of SO₄-S [Stevenson, 1986]. This finding is in accordance with the fact that the soils amended with the SS, DW and PL [C: S ratios, 23-54: 1] showing the S mineralization throughout the incubation period. The addition of the rice straw, whose C: S ratio [36: 1] was above the critical range [200-400: 1] showed S immobilization throughout the incubation period. So, we propose that the addition of an OM with a high C: S ratio, which can induce the immobilization of SO₄-S, increases the amount of microbial biomass-S that persists throughout the incubation phase. On the other hand, the addition of an OM with a low C: S ratio does not lead to a substantial increase in the amount of soil microbial biomass-S.
Howlader et al.

Conclusions

Rice straw, which has a higher C: N ratio \( \frac{67}{1} \) induced the highest rate of CO\(_2\)-C evolution, but showed a slower decomposition than that of the dustbin waste, poultry litter and sewage sludge with lower C: N ratios \( \frac{8-20}{1} \). Rice straw with a C: N: S ratio of 636: 9: 1 contributed a higher amount of biomass-C, -N and -S than that of sewage sludge, poultry litter, dustbin waste or fertilizer. The higher percentages of nitrogen and sulfur were assimilated by soil microbial biomass from added rice straw more than that of the sewage sludge, poultry litter, dustbin waste and fertilizer. The soils amended with the sewage sludge, dustbin waste and poultry litter showed nitrogen and sulfur mineralization but immobilization of nitrogen and sulfur were observed in the rice straw amended soil.

References


Russel, D.F. 1986 MSTAT Director. Crop and Soil Science Department, Michigan State University, USA.


