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SOIL CARBON AND NITROGEN DYNAMICS IN DEGRADED SOILS RECLAIMED USING STABILIZED ORGANIC AMENDMENTS

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ABSTRACT

Organic carbon (C) and nutrients in manure can enhance the quality of degraded soil if they are sequestered in the soil. This improvement could be facilitated by applying manure mixed with a high carbon material or by composting the manure. To identify labile and stable nitrogen (N) and carbon C fractions in degraded soil connected to different treatments, a lab incubation experiment was conducted. Treatments included lime plus mineral fertilizer, two levels of compost and two levels of manure combined with paper mill sludge to achieve C:N ratios of 20:1 and 30:1. The treatments were added to degraded soil and immediately incubated. Data revealed that using organic amendments instead of lime and fertilizer was more effective in creating large stable N and C fractions, proving that the N and C sequestered by both C:N adjustment techniques is much higher than the N and C pools linked to inorganic fertilizer. However, paper mill sludge and manure exhibited higher levels of microbial biomass, which could lead to improved long-term nutrient cycling. It doesn't seem that adding more paper mill sludge to raise the C:N ratio from 20:01 to 30:1 was beneficial. According to these findings, composting and mixed manure + paper mill sludge amendments were equally successful in creating stable N pools in degraded soil.

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INTRODUCTION

The application of organic amendments to degraded soil is probably the most successful reclamation technique because of its widespread availability, inexpensive, and suitable for recovering organic waste **(Wang *et al.*, 2014; Aboukila, 2019)**. When organic amendments are added to degraded soil, the microbiological and enzymatic activities of the soil are restored, improving soil aggregation and soil structure **(Lakdhar *et al.*, 2009; Aboukila *et al.*, 2018).** Soil aggregation developments improve soil permeability, porosity, and water infiltration **(Haynes and Naidu, 1998)**.

The quality of water can be adversely affected by mineralization and subsequent leaching losses of soil-labile nitrogen (N) pools. Microbial cycling needs to transform N into pools of more stable N for soils to be able to sequester it **(Paré *et al.*, 1998; Kaye *et al.*, 2002a)**. Furthermore, in nutrient-depleted degraded soils, microbial activity is essential for maintaining nitrogen and reestablishing nutrient cycling because it significantly reduces inorganic N loss **(Davidson *et al.*, 1992; Stark and Hart, 1997)**. In addition to having very low nutrient contents, degraded soils could be highly capable of retaining N if sufficient C is available to support microbial activity **(Ingram *et al.*, 2005; Abdelraof *et al.*, 2023)**.

Compost application has been shown to enhance soil quality and reduce nitrogen loss through leaching **(Bernal *et al.*, 1998; Amlinger *et al.*, 2003; Aboukila and Nilahyane, 2022)**. Compost incubation research revealed that improvements in soil bulk density, porosity, aggregate stability, hydraulic conductivity, and water retention capacity, were all correlated with the compost application rate **(Aggelides and Londra, 2000; Aboukila *et al.*, 2018)** Long-term soil microbial activity was enhanced by adding fresh manure, and less processed materials, like sewage sludges, may offer a better source microbial food **(Gigliotti *et al.*, 2002)**. However, applying fresh manure exposes large levels of inorganic N to leaching, indicating that manure alone has limited ability to hold nitrogen in a stable form **(Carpenter *et al.*, 1998)**.

On the other hand, it has been demonstrated that combining manure with a high carbon source can reduce N leaching loss and enhance soil characteristics. Although it seems that applying these materials together greatly lowers the labile N fraction, it is uncertain how this affects the stable N and C fractions and how much microbial activity is maintained when fresh manure is applied instead of compost. Incubation studies are useful for investigating C and N changes and the creation of stable and labile C and N fractions since they are conducted in the absence of plants or environmental components **(Hart *et al.*, 1994).** This method was used to design a laboratory incubation experiment to investigate the differentiation between labile and stable N and C fractions right after amendment application.

In this study, degraded mine soil was treated with amendments in the lab, and the mixture was then incubated for a year. The study aimed to optimize mineralization and determine the potential for inorganic N leaching from degraded soils treated with lime and fertilizer, compost, or fresh manure combined with paper mill sludge. Furthermore, evaluating stable N and C fractions could evaluate these treatments' capacity for holding nutrients over time, whilst measuring microbial biomass would provide information on the microbial activity linked to these treatments. Additionally, determining the microbial biomass fraction would aid in predicting the long-term nutrient cycling restoration in soils treated with these amendments.

MATERIALS AND METHODS

Soil Location and Sampling

Soil samples for the laboratory incubation experiment were collected from a degraded mine land site in Schuylkill County, Pennsylvania, USA. The annual precipitation at this location was 132 cm. Soil samples were collected using soil cores (5 cm deep and 4 cm in diameter). Soil samples were composited and sieved with a 0.75 cm sieve. Sub-samples were analyzed for selected physical and chemical properties (Table 1).

Amendments

Five types of amendments were applied in this study included ground agricultural limestone, inorganic fertilizer (as NH_4NO_3, triple super phosphate (TSP), and KCl), poultry manure, paper mill sludge (PMS), and composted poultry manure as reclamation amendments. The characterizations of the organic amendments were calculated using the methodology described in **Kehres (2003)**. Table 2 shows the characteristics of organic materials.

For a total of six different treatments with three replications, field moist soil samples were mixed with amendments as per quantities indicated in Table 3. Treatments included lime and inorganic fertilizer (IF), two rates of composted poultry manure (C1 and C2), two rates of paper mill sludge (PMS) mixed with poultry manure (M20 and M30) to give C:N ratios of 20:1 and 30:1, and a soil with no amendment added as a control (Ctrl).

A dry weight total of 150 grams of materials per experimental unit was used to calculate the portions of each material. Materials were mixed 1 day before the start of the incubation experiment. A subsample of the soil and all amendment materials was dried at 105 °C for 2 days to calculate the moisture content.

Laboratory incubation experiment

To separate labile and stable N fractions, a long-term aerobic incubation experiment was conducted under controlled conditions and repeated leaching to remove labile N **(Kaye *et al.*, 2003)**. This method permits the assessment of inorganic N possibly accessible for plant uptake or loss by leaching while simultaneously measuring the microbially relevant C pool **(Robertson *et al.*, 1999)**. After the mixing treatments with soil, the incubated soils were leached at day 1, 6, 13, 22, 34, 49, 64, 80, 99, 118, 139, 169, 201, 230, 265, 299, 334, 365 and 392. Using the method described by **Nadelhoffer (1990)**.

Table 1. Initial characterizations of the soil used for experimentation.

Analyte	
pH	5.1
Bulk density	1.77 g cm^{-3}
Texture	Sandy loam
Phosphorus (P)	5.05 mg kg^{-1}
Potassium (K)	41.85 mg kg^{-1}
Magnesium (Mg)	65.45 mg kg^{-1}
Acidity	9.15 meq100 g^{-1}
CEC	11.08 meq100g^{-1}
Total nitrogen (N)	0.09 %
NO_3-N	2.50 mg kg^{-1}
Total Carbon (C)	31.8 g kg^{-1}
Organic Carbon	30.9 g kg^{-1}

Table 2. Chemical analysis of amendments used in the experiment.

Analyte	**Composted manure**	**Fresh manure**	**Paper mill sludge**
pH (1:1 w:w)	8.1	8.3	7.3
EC (dS/m, 1:5 w:w)	5.81	19.20	1.15
Moisture (gravimetric %)	55.0	53.2	61.8
Organic Matter (%)	57.8	49.2	46.1
Total N (%)	2.70	4.20	0.20
Organic N (%)	2.70	2.9	0.20
Ammonium N (mg/kg)	532.8	13586	9.8
Carbon (%)	34.5	30.9	26.2
C:N Ratio	12.5	7.3	126.0
Phosphorus as P_2O_5 (%)	3.07	4.77	0.09
Potassium as K_2O (%)	2.16	2.85	0.03

Table 3. Amendments application rates to degraded soil in each experimental unit. Each cup was filled with a total of 150 grams dry weight bases.

Treatment		Material (g)
Control	**Ctrl**	
Soil		150
Inorganic Fertilizer	**IF**	
lime		8.82
NH_4NO_3		0.25
TSP		0.79
KCl		0.29
Soil		139.9
Compost 1	**C1**	
compost		14.9
soil		135.1
Compost 2	**C2**	
compost		27.2
soil		122.8
Paper mill sludge+Manure	**M20**	
paper mill sludge		17.7
poultry manure		8.80
soil		123.5
Paper mill sludge+Manure	**M30**	
paper mill sludge		27.5
poultry manure		8.80
soil		113.7

Collected leachate was analyzed for total soluble C and organic C by combustion, and for inorganic N (NH_4^+ and NO_3^-) by flow injection colorimetry. To remove any unleached inorganic N, at the end of the experiment (392 d), a subsample of soil (10-gram dry weight bases) was extracted using 50 ml of 0.5 M K_2SO_4 **(Motavalli *et al.*, 1995)**.

Labile N was defined as the sum of inorganic N (NH_4^+ + NO_3^-) leached and the inorganic N extracted with K_2SO_4. Stable N was determined by deducting the labile N from the initial soil N **(Kaye *et al.*, 2002b).**

At the end of the experiment, microbial biomass was assessed for each soil by the chloroform-fumigation extraction approach (Brooks et al., 1985). To quantify microbial biomass, the total extracted nitrogen measured post-fumigation was subtracted from the total nitrogen extracted prior to fumigation **(Brooks *et al.*, 1985)**.

One day before each leaching event, soil respiration was quantified by measuring the amount of CO_2 that accumulated over the course of six hours in each incubation soil cup sealed in a 1 L plastic jar using a LI-COR CO_2/ H_2O Analyzer (LI- 7000) **(Robertson *et al.*, 1999; Kaye *et al.*, 2002b)**. Labile carbon was computed by summing up the carbon losses from respiration and leaching. Stable C was calculated by deducting labile carbon from initial carbon.

Statistical analysis

For mean differentiation, Fisher's least significant difference (LSD) was computed after an analysis of variance (ANOVA) was calculated using SAS 13.1 statistical software (SAS Institute, 2013), to check for statistical differences.

RESULTS AND DISCUSSION

Nitrogen

In the first 5 weeks of incubation, the incubation experiment was marked by rapid decrease of NO_3 -N, NH_4 -N, and labile N (NH_4^+ + NO_3^-) with significantly lower inorganic nitrogen leaching losses afterwards (Fig. 1, 2, and 3). Based on leaching trends throughout the experiment, the leaching losses can be split into three distinct periods: 0-48, 49-228, and 229-392 days. The bulk of losses of the NH_4^+ fraction began rapidly, and by day 33, they had drastically decreased (the fifth

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leaching event) (Fig. 1). During the first 4 leaching events (by day 21), both PMS+manure treatments (M20 and M30) and the inorganic fertilizer (IF) treatment lost a significant amount of N in the form of NH_4^+. Conversely, the compost treatments lost comparatively little NH_4^+. In contrast, the greatest amount of NO_3^- leaching occurred during the first 6 leaching events (by day 48) with the compost (C1 and C2) and inorganic fertilizer (IF) treatments (Fig. 2). During this period (by the day 48 of incubation), these three treatments (IF, C1, and C2) leached 78 to 113 times more NO_3^- than the PMS+manure treatments (M20, and M30).

Starting from the 6th leaching event (day 49) ammonium losses were far smaller than the first 5 leaching events. Compared to the other treatments, the M30 treatment leached a little bit more NH_4^+ (Fig. 1). The second period exhibited a significant increase in NO_3^- leaching from the M20 and M30 treatments, which was three times higher than that from the inorganic fertilizer and compost treatments (Fig. 2). Compared to the first two periods, nitrate leaching losses were significantly lower in the last six leaching events (day 229 to the end of experiment). Although the M20 treatments lost 2 times NO_3^- than the compost treatments, the treatment differences were also less than in the first two periods.

This study demonstrates the behaviour of labile N fractions of different treatments. Throughout period one, easily available N was rapidly mineralized till the pool was depleted, at which point inorganic nitrogen leaching losses reached zero. A more resistant pool of N started to mineralize around day 66 and was almost completely consumed by day 292; more resistant N then progressively mineralized (Fig. 3). A more resistant fraction of N started to mineralize after day 66 (Fig. 3) and was almost completely depleted by day 292; after that, more resistant N was slowly mineralized. The reduced rates of nitrogen loss, from compost treatments, throughout the 2nd and 3rd period indicate a bigger fraction of more resistant N that gradually mineralized during the trial. **Gordillo and Cabrera (1997)** found that a first-order kinetics model could be used to separate N mineralization into two pools: a rapid pool and a slow pool. This was based on the data of a 5-

month incubation of poultry manure. As a result, the experiment's findings about the N pools' varying resistance to mineralization seem realistic.

It's interesting to note that throughout the year, there were noticeable variations in the amounts and timing of NH_4^+ or NO_3^- lost via leaching. The form in which nitrogen was added with these amendments did not significantly affect the total leaching loss of labile nitrogen in organic amended soils (Fig. 4, and 5). These findings indicate that organic treatments have an equal chance of N mineralization and consequent loss of inorganic N to leaching under the rigorous leaching regimen of the incubation. In comparison to the initial soil N at the beginning of the trial, the organic treated soils lost 16 to 28% of the initial N (Fig. 5). The treatment with the greatest beginning N, C2, lost just 16% of its start N, whereas C1 lost 21% (Fig. 5). Although starting with 1.6 times more nitrogen than the lowest rate of compost (C1), C2 lost less nitrogen, indicating compost's ability to reduce N leaching at high application rates. During the trial, both PMS+manure treatments (M20 and M30) lost the same amount of nitrogen as lowest rate of compost (C1) (Fig. 4), indicating that fresh manure combined with paper mill sludge has the ability to limit leaching losses as effectively as compost having the same amount of nitrogen. In contrast, the IF treatment lost 52% of the initial N, indicating that the N pool was significantly depleted in this treatment. A similar finding was reported by **Cabrera *et al.* (1993)** in a 35-day incubation investigation using poultry manure.

At the conclusion of the incubation trial, there were significant stable N pools in the organic treatments (Fig. 4). As anticipated, C2 resulted in the biggest stable N pool, which coincided with the increased N addition. It was noticeable the similarity in stable N pool size between the first rate of compost (C1) and both PMS+manure treatments (M20 and M30). These findings show that, when the initial N was equal, PMS+manure was equally effective as compost at expanding the stable nitrogen fraction. Furthermore, a C:N ratio of 20:1 was used to accomplish this rise in the stable N pool, indicating that the extra C used in the 30:1 treatment was not required to reduce leaching or raise stable N.

These findings are in line with research that has been done on the insertion of N_{15} tracers to forest soils to observe stable and labile pools. That research has demonstrated that stable pools remain significantly bigger than labile pools even following intensive leaching regimes. According to a one-year lab incubation research conducted by **Kaye *et al.* (2002a),** Over 2/3 of added nitrogen was sequestered in plantation soils from Puerto Rico. Similarly, **Nadelhoffer *et al.* (1999)** reported roughly 75 to 80% of added N was retained in soils with N_{15} tracer applications in two different field investigations. Additionally, these investigations found a clear relationship between N added and stable N fractions, which was also confirmed in this study.

The (IF) application generates a significantly less stable N pool. These findings suggest that using inorganic fertilizer would not be an appropriate strategy to store nitrogen and enhance ecosystem performance. It is interesting to note that the stable N fraction in the control soil (Ctrl) was larger than in the inorganic fertilizer treatment (IF). This suggests that the addition of inorganic fertilizer resulted in a smaller stable N pool than if no amendment had been added to the soil due to the intense leaching conditions of this experiment. **Marinari *et al.* (2000)** conducted a field experiment in which they applied NH_4NO_3 to sandy clay loam soil. They found that the high input of N activated native soil organic matter, providing a nutritional source for microbial biomass, and lead to increased CO_2 generation and nitrogen mineralization.

Microbial biomass, a component of the stable N pool, was twice as large in the M30 treatment as in the M20 treatment (Fig. 6). The PMS+manure treatments had 4 to 11 times more microbial biomass than the compost treatments. The much bigger microbial fractions in the PMS+manure treated soil indicate that more microbially accessible C may survive after one year of severe leaching than in compost treatments. **Aboukila *et al.* (2018)** conducted an incubation study to demonstrate the soil benefits of adding brewers' spent grain to degraded soils rather than compost, finding that soil organic matter, soil water holding capacity, macronutrients, micronutrients, and germination parameters were enhanced in soils amended with fresh material. As a result, using PMS+manure instead of compost could have a significant impact on the soil's sustainability and long-term nutrient cycling.

Despite the compost (C1, C2) having fewer microbial biomass than the PMS+manure (M20, M30) treatments (Fig. 6), they had significantly bigger fractions than the IF and Ctrl soils (1.7 to 4.6 times more). Given the huge and small pools of stable N and C related to the organic and inorganic treatments, respectively, the variation in microbial biomass between the organic compared to the IF treatment was not surprising. Furthermore, there was no difference in microbial biomass among IF and Ctrl, indicating that the IF treatment did not lead to a significant increase in the microbial pool.

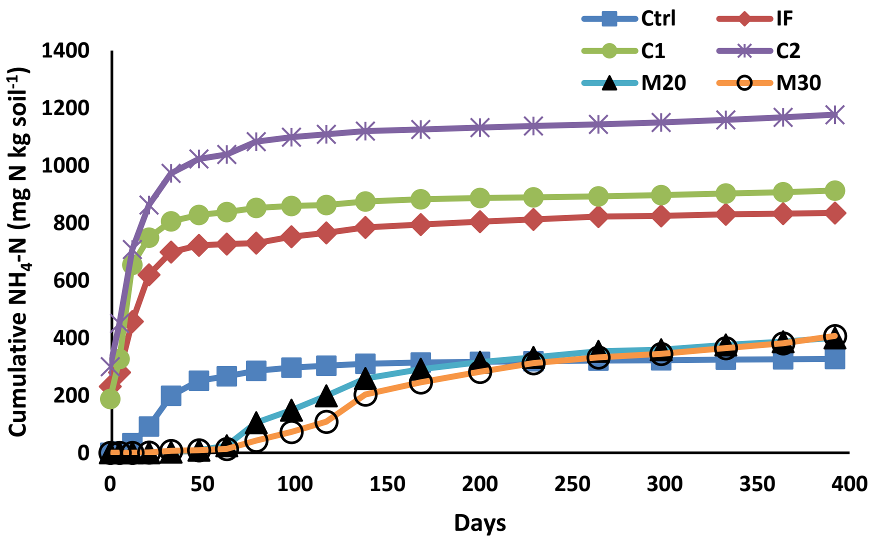

Figure 1. Cumulative NH_4^+leached during the incubation experiment.

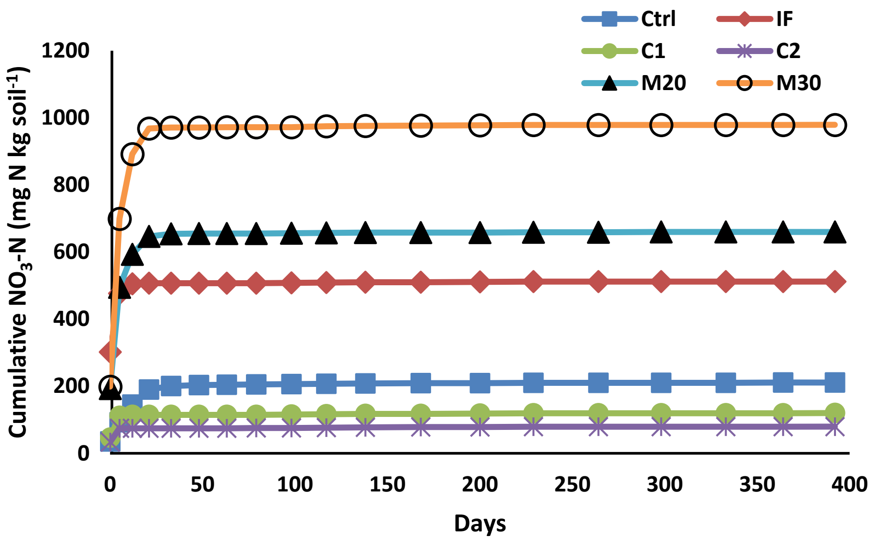

Figure 2. Cumulative NO_3^- leached during the incubation experiment.

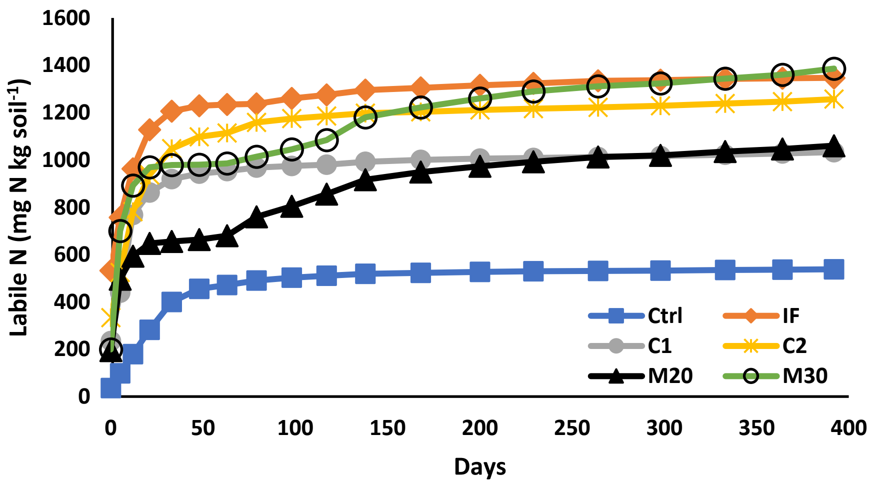

Figure 3. Cumulative Labile N (NH_4^+ and NO_3^-) leached during the incubation experiment.

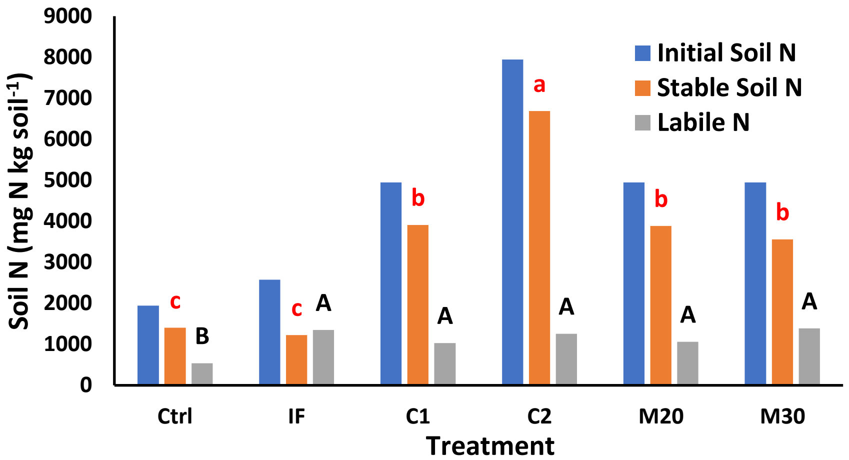

Figure 4. Initial, stable, and labile soil N by treatment in the incubation experiment.

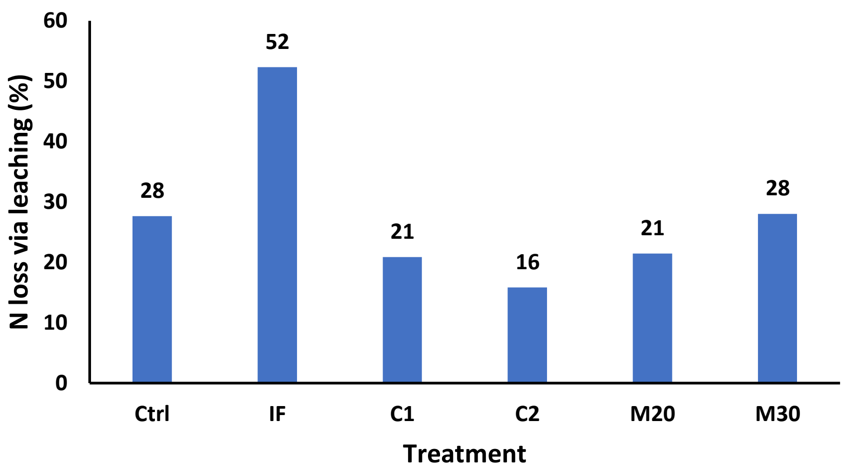

Figure 5. Labile N ($NH_4^+ + NO_3^-$) loss via leaching (%) by treatment in the incubation experiment.

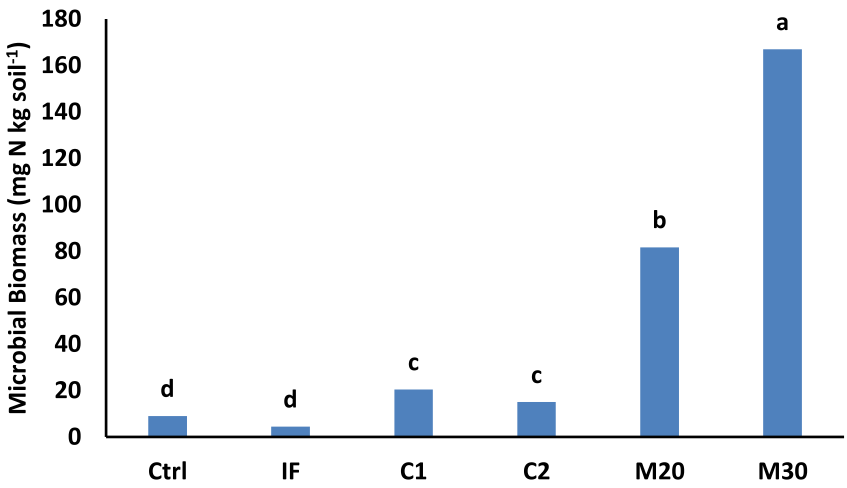

Figure 6. Microbial biomass N by treatment in the incubation experiment.

Carbon

Leaching did not represent a substantial loss mechanism in this trial because the losses from all treatments were negligible in comparison to the initial carbon (Fig. 7, 8). The majority of C leaching happened within the first 12 days of incubation, then tiny amount carbon leaching from all treatments after that (Fig. 7). Throughout the incubation period, PMS+manure treatments (M20 and M30) lost about 5.5 times more carbon through leaching than compost treatments (C1 and C2), which lost only marginally more carbon than IF and Ctrl treatments. Composting process significantly reduces labile C, which explains the huge variations in C leached across PMS+manure and compost amended soils, although comparable great starting C inputs **(Hanselman *et al.*, 2004)**.

CO_2 fluxes quantified the potential for respiration of amended soils and represented nearly the whole labile C pool (Fig. 9). For the

PMS+manure treatments, respiration rates reached its highest point on day 12, were decreased in the next 124 days, and then steadied at comparatively low rates for the rest of the study (Fig. 9). Throughout the first 117 days of the trial, respiration losses from PMS+manure were higher than those from all other amendments; compost treatments did not show an increased respiration level of C loss throughout the same duration. In an incubation study of a silt loam soil mixed with manures at different stages of composting, **Bernal *et al.* (1998)** reported that the highest rates of C respiration were found in fresh, unstabilized manures as was the case in this investigation. Regardless of the C:N ratio, both PMS+manure (M20, M30) treatments lost 36% of their initial C via respiration (Fig. 10). These increased respiration levels indicate the presence of readily mineralizable C, implying that the form of carbon in the PMS is not so resistant that it is instantly inaccessible to microorganisms **(Paré *et al.*, 1998)**.

Compost treated soils lost 5.3 - 7.22% of their starting carbon by respiration (Fig. 10), although having 1.5 to 2.5 times more initial carbon than the IF treatment (Table 3). Labile C mineralization during the composting procedure could explain the comparatively low respiration losses from C1 and C2 amended soils **(Flavel and Murphy, 2006)**. The respiration losses from the Ctrl and IF treatments were found to be much lower than those from the organic treatments. Both respiration losses accounted for approximately 6% of the initial carbon and indicated a decrease in the activity of microbial populations in both treatments.

Stable C fractions did not correspond as well with amounts of organic C added as did stable N pools (Fig. 11). Once again, the biggest stable C pool was seen with the C2 therapy. Despite beginning with 22% less C than the M30. Even though the M20 treatment started with 1.3 times more carbon than the C1 treatment, by the end of the incubation, it had a lower pool of stable carbon. Furthermore, the IF treatment produced a bigger stable carbon fraction than the Ctrl. Regarding the stable C, the C:N ratio seems to be more important for the PMS+manure amendments than it was for stable nitrogen.

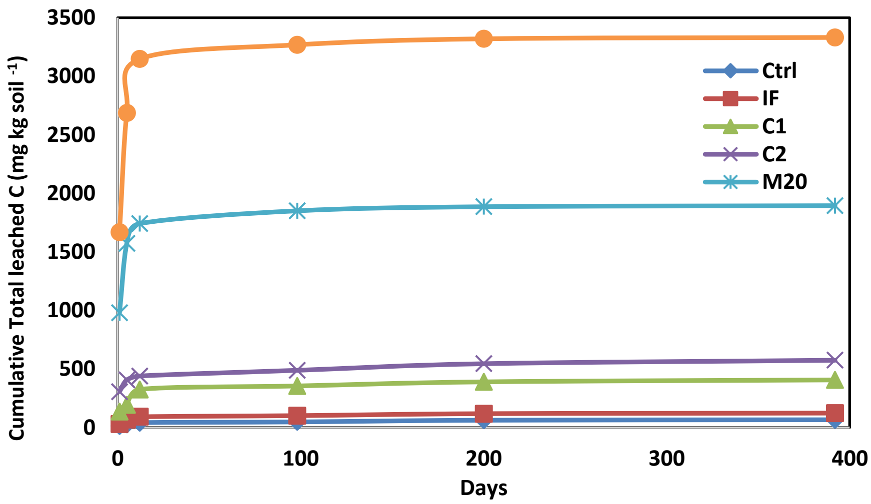

Figure 7. Cumulative total C leached during incubation experiment.

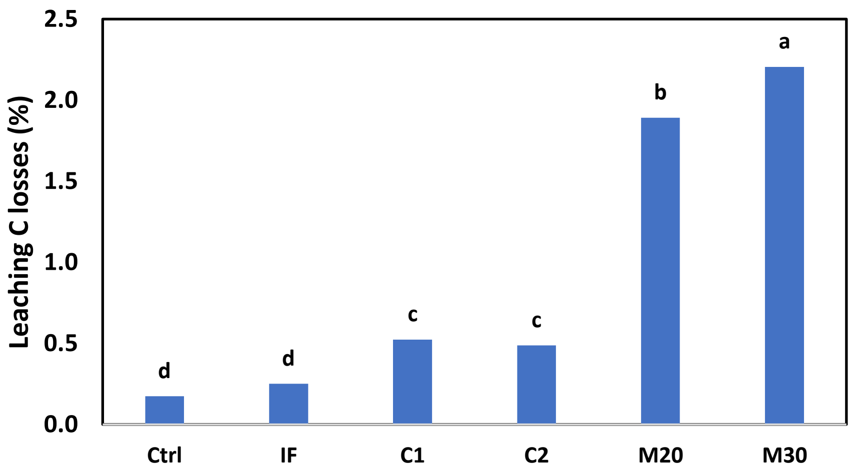

Figure 8. Leaching C losses (%) by treatment during the incubation experiment.

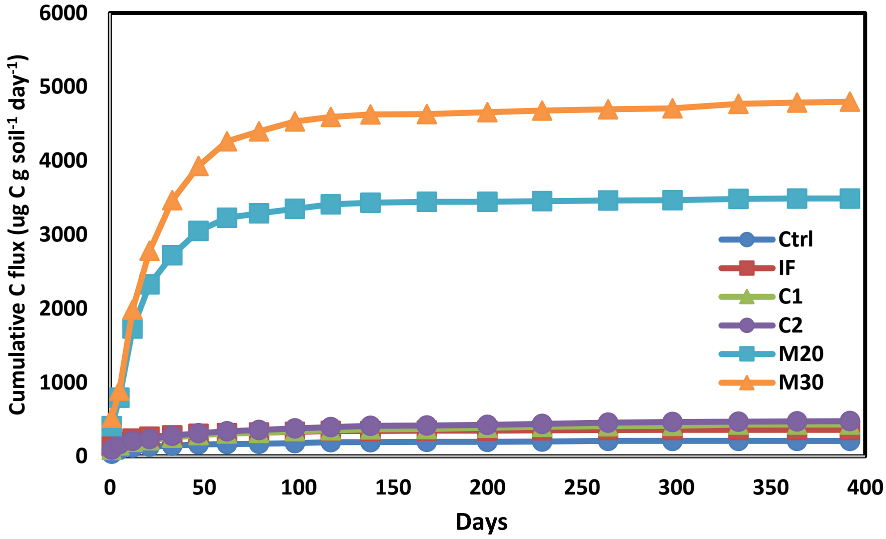

Figure 9. Cumulative carbon fluxes during the incubation experiment.

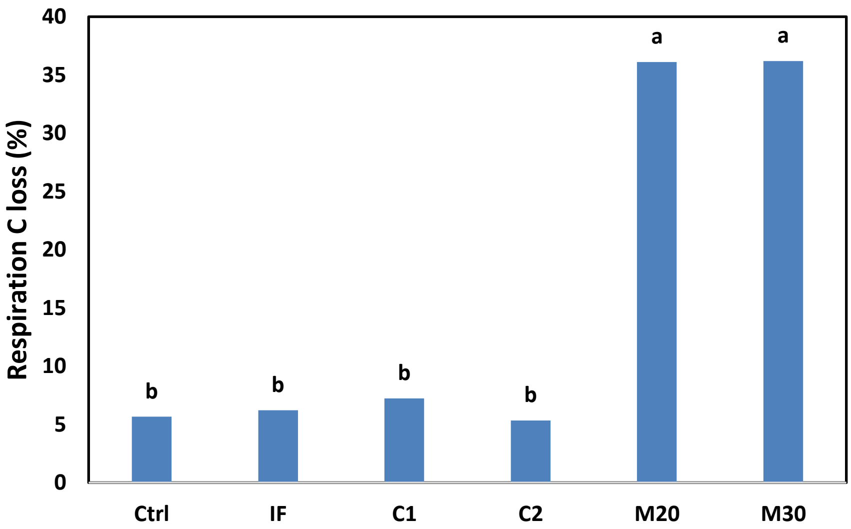

Figure 10. Respiration C loss (%) by treatment during the incubation experiment.

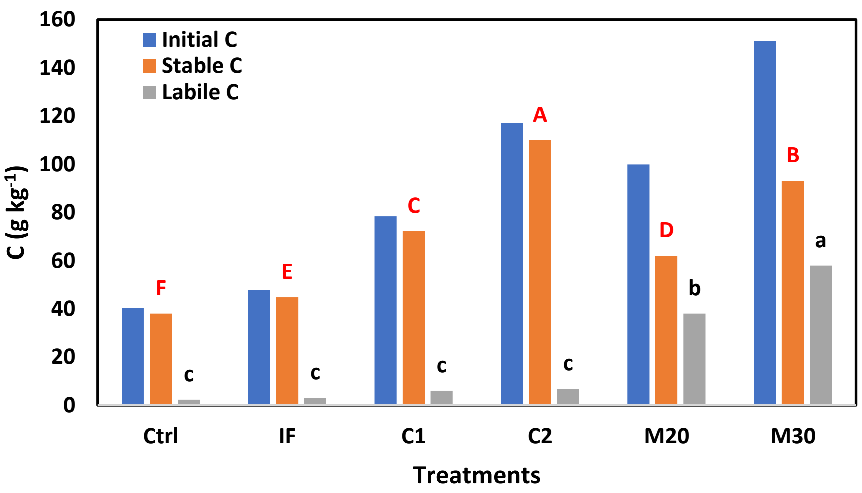

Figure 11. Initial, stable, and labile C pools by treatment in the incubation experiment.

Conclusions

Differentiating between the stable and labile N and C pools linked to different treatments was successfully accomplished through the lab incubation experiment. Whether manure was applied alone or in combination with paper mill sludge, organic treatments were significantly more successful in creating sizable, stable pools of N and C. As anticipated, the addition of compost, particularly at a higher rate (C2), significantly reduced the amount of inorganic N leaching and created sizable N and C pools that were directly correlated with the amount of N and C added. More noteworthy, though, was the fact that mixing manure and paper mill sludge, regardless of the C:N ratio, produced large microbial pools, minimized N leaching, and built large stable N and C pools just as well as compost. Furthermore, compared to compost treatments, these treatments seem to be associated with higher levels of microbial activity, which may result in improved long-term soil nutrient cycling. Applying PMS+manure directly to the soil may decrease N and C loss from the original organic materials, while increasing the potential sequestration of N and C from those materials, when taking into account the C and possible N losses caused during the composting process. Finally, since the stable N pools and N leaching losses did not differ measurably, it doesn't seem that adding more PMS to raise the C:N ratio from 20:1 to 30:1 was beneficial.

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الملخص العربي

ديناميكية الكربون والنيتروجين في الأراضي المتدهورة والمستصلحة بمصلحات عضوية متوازنة

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الكربون العضوي والعناصر الغذائية الموجودة في روث الحيوانات لها القدرة على تحسين جودة الأراضي المتدهورة بشرط ان يتم الاحتفاظ بها في التربة. يمكن زيادة قدرة التربة على الاحتفاظ بالعناصر من خلال خلط روث الحيوانات مع مادة عالية المحتوى في الكربون او عن طريق تحويل روث الحيوانات الى كومبوست. تم تنفيذ تجربة تحضين معملية للتعرف على صور النيتروجين والكربون القابلة والمقاومة للتحلل والمرتبطة بمعاملات مختلفة في أراضي متدهورة، اشتملت المعاملات على جير وسماد معدني، معدلين من الكومبوست، ومعدلين من روث الحيوانات المضاف اليه مخلفات صناعة الورق وذلك للحصول على نسبة كربون الى نيتروجين 20:1, 30:1. تم التحضين مباشرة بعد إضافة المعاملات الى الارض المتدهورة. أوضحت النتائج ان استخدام المصلحات العضوية بدلا من الأسمدة المعدنية في استصلاح الأراضي كان أكثر كفاءة في تخليق كمية كبيرة من الكربون والنيتروجين المقاوم للتحلل، مما أدى الى ان تكون كمية النيتروجين والكربون المحتفظ به في التربة أكبر بكثير من مثيلاتها في حالة استخدام الأسمدة المعدنية. من ناحية أخرى، وجد أكبر مستوى من الكتلة الحيوية الميكروبية في معاملات روث الحيوانات المختلطة بمخلفات صناعة الورق، مما قد يؤدي إلى تحسين دورة المغذيات على المدى الطويل. لا يبدو أن إضافة المزيد من مخلفات مصانع الورق لرفع نسبة الكربون : النتروجين من 20:01 إلى 30:1 كان مفيدًا. وفقًا لهذه النتائج، فان طريقتي الكومبوست والروث المختلط بمخلفات مصانع الورق ناجحة بنفس القدر في إنشاء كمية كبيرة من النيتروجين الثابتة في الأراضي المتدهورة.