



## Carbon Dioxide Release as an Index of Mineralization Rates of Organic Substrates

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**Abstract** Mineralization of nutrients from organic materials is vital for optimum plant growth and development. Various methods have been used to evaluate the mineralization rate of different organic substrates. Of these, carbon dioxide release is a reliable method to estimate mineralization rate. Four different substrates: dry chicken manure, *Azolla*, coconut coir dust (CCD), and *Gliricidia sepium* leaves and the combination of these substrates with SNAP solution on their rates of mineralization was determined. The results were significantly different in the amounts of CO<sub>2</sub> released from the substrates. CO<sub>2</sub> release from the substrates was stimulated by SNAP. Among all treatments, *G. sepium* with SNAP yielded the highest amount while coconut coir dust gave the lowest. Nitrogen mineralized to about fifty percent (50%) during the first week of all treatments. Using soil organic materials such as *G. sepium* leaves would quickly provide the soil with more mineralized nutrients which are available for plant growth and development.

**Keywords** mineralization, CO<sub>2</sub> release, organic substrates, *Gliricidia sepium*

### INTRODUCTION

Mineralization is an important process which regulates the nutrient cycle (Curtin et al., 1997). It is also a continuous process which is controlled by many factors such as temperature (Curtin et al., 1997; Crohn, 2004; Gaskel and Smith, 2007; Li and Li, 2014), moisture (Curtin et al., 1997; Gaskel and Smith, 2007), pH (Curtin et al., 1997; Ouyang et al., 2008), microorganisms (Frankel and Bazylnski, 2003; Allison et al., 2009) or organisms of higher class such as earthworms (Coúteaux et al., 1995), herbivores (Schrama et al., 2013), soil properties such as clay types (Deenik, 2006), substrate quality and quantity (Howard and Howard, 1993; Eiland et al., 2001; Gaskell and Smith 2007; Li and Li, 2014), and the cultivation practices.

Understanding the processes involved in mineralization, and the factors that affect mineralization rate, would help estimate the right type and amount of mineralized material to guarantee optimum yield

and minimize the adverse effects of the over application of nitrogen (N) sources (Pereira et al., 2006; Hartz et al., 2000; Crohon 2004; Bordilio et al., 2013; Li and Li, 2014).

The contents of carbon dioxide (CO<sub>2</sub>) determine the rate of mineralization by measuring the trapped CO<sub>2</sub> in the alkali solution, such as Ca(OH)<sub>2</sub>, NaOH or KOH and titrate it against hydrochloric acid (HCl) with addition of barium chloride (BaCl<sub>2</sub>) (Peirera et al., 2006; Hartz and Britton, 2003; Makende and Ayeni, 2013).

## OBJECTIVE

The objectives of this study were to estimate the mineralization rates of different organic materials using the CO<sub>2</sub> release as an index, and to examine the effects of nutrient additions on the mineralization rates of these materials.

## METHODOLOGY

Soil samples (Alipit Clay Soil) were collected from an unfertilized upland area of the U.P. Los Baños Central Experiment Station. The soil was air-dried, cleaned from vegetative material and passed through a 2.0 mm sieve. Fifty (50) grams of this sieved soil was placed inside incubation jars and mixed with 0.125 g of each nutrient source (5 t ha<sup>-1</sup>). Complete Randomized Design (CRD) with three replications was used in this experiment. The treatments were: Control (CT)-soil alone with no additional nutrients, dry chicken manure (CM), coconut coir dust (CCD), *Gliricidia sepium* (GS), Azolla (AZ), simple nutrient addition program (SNAP) nutrient solution, CM + SNAP, CCD + SNAP, GS +SNAP and AZ + SNAP. The incubation study was conducted from April to May 2014 in the Soil Fertility Laboratory of the Agricultural Science Cluster (ASC), College of Agriculture, U. P. Los Baños, Philippines.

The Simple Nutrient Addition Program (SNAP) nutrient solution was formulated by the Institute of Plant Breeding, College of Agriculture, U.P. Los Baños. The solution was prepared by combining 20 ml of SNAP A and 20 ml of SNAP B, and adding this to one liter of distilled water. Forty (40) ml of the prepared SNAP solution mixture was added to each SNAP-treated jar.

### Determination of Carbon Dioxide Release and Mineralized Nitrogen

Mineralization rate was measured by determining the amount of CO<sub>2</sub> released from the different treatments. The incubation jar set-up was prepared prior to the start of the experiment. Prior to application, the substrates were grounded and oven-dried overnight at 40°C. The appropriate amount of residue was added to 50 g soil at a rate of 0.125 g jar<sup>-1</sup>. Based on the amount of soil, this application rate approximates to 5 t ha<sup>-1</sup>, the amount of residue applied in many farming systems. For the control treatment, no organic material was added in the same amount of soil. The experiment was conducted for the duration of approximately 8 weeks.

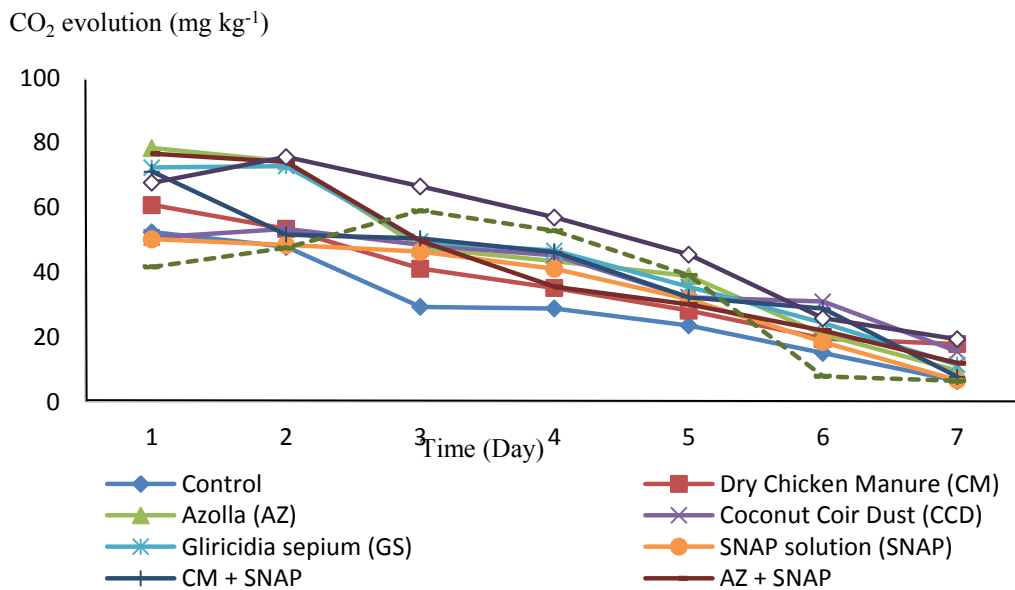
The mixtures of soil and treatment nutrient sources were supplied with moisture at field capacity. A 50 mL beaker containing 30 mL of 0.3 M NaOH was placed in the center of the jar to trap the CO<sub>2</sub> released from each treatment. The trapped CO<sub>2</sub> was then transferred to a 100 mL beaker, and 3 drops of phenolphthalein were added before titration with 0.2 M HCl.

## RESULTS AND DISCUSSION

The incubation experiment examined the mineralization rate of different organic materials over a 7-day period. Results showed that there were significant differences between treatments. The highest amount

of CO<sub>2</sub> released on the first day was measured from Azolla and AZ + SNAP with 78.3 and 76.6 mg kg<sup>-1</sup> soil, respectively. These treatments were closely followed by *G. sepium* and CM + SNAP with 72.3 and 70.9 mg kg<sup>-1</sup> soil respectively. Since the measured CO<sub>2</sub> from these four treatments were ranked closely, differences of the CO<sub>2</sub> released were found to be insignificant.

The measured CO<sub>2</sub> on the second day showed that soft, fresh materials with high source of N continuously exhibited faster rate of mineralization when compared with other organic materials. Treatments with Azolla and *G. sepium* demonstrated the highest amount of CO<sub>2</sub> with 74.2 and 72.7 mg kg<sup>-1</sup> soil, respectively. The addition of nutrients hastened the mineralization rates as shown by Azolla + SNAP and GS+ SNAP. Similar effects were observed from Day 3 to Day 7 as those treatments with SNAP recorded high amount of CO<sub>2</sub> release. The *G. sepium* + SNAP Treatment dominated other treatments and their differences were found to be significant. Its performance is clearly presented in Fig. 1. Treatments with Azolla and *G. sepium* alone ranked second and third. It is also interesting to note that coconut coir dust played second on Day 3 to 4. Fontaine et al. (2003) reported that the increase in the amount of CO<sub>2</sub> released in the first week for all treatments may be attributed to the activities of microorganisms attacking the labile and readily decomposable substrates such as sugar, starch, and cellulose. The surge of CO<sub>2</sub> released from the soil in the first day (Fig. 1) might due to rewetting of the soil which allowed the surviving microorganisms to immediately attack SOM and the substrates (Keift et al., 1987). The control, although having a lower total CO<sub>2</sub> release, it had a starting point which was also as high as chicken manure and SNAP solution. The soil had high SOM content (6.3%) could increase mineralization.



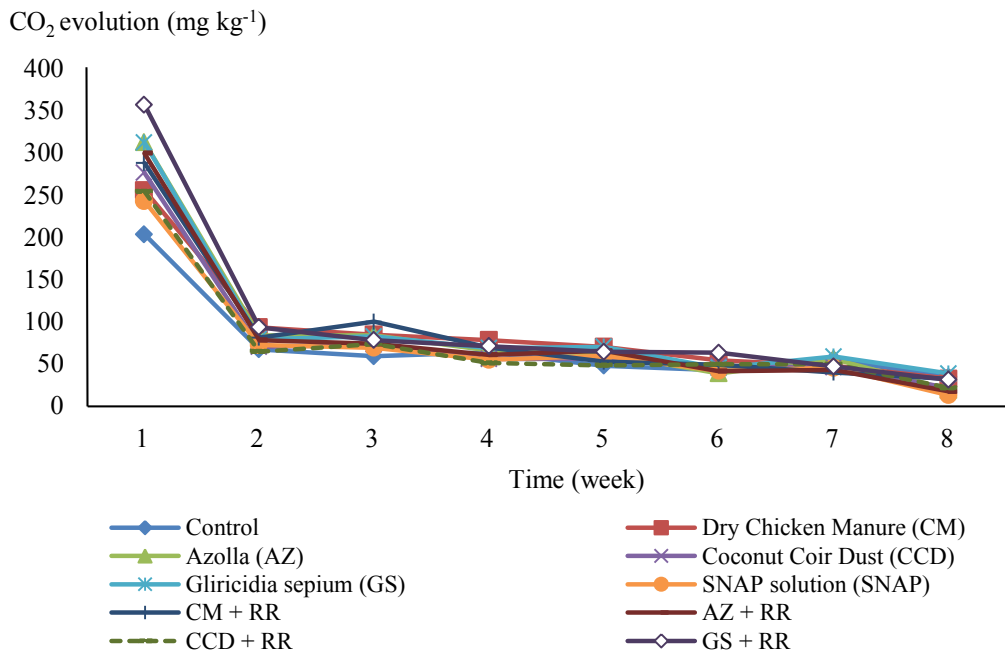
**Fig. 1 Daily carbon dioxide (mg kg<sup>-1</sup>) released from different organic substrates over the period of seven days**

Franzluebbers et al. (1994) concluded that N mineralization can be related to the amount of SOM in the soil. Another study of Chuwdhury et al. (2014) on the effect of malic acid addition to CO<sub>2</sub> release found that treatments with or without nutrient addition had very high CO<sub>2</sub> production from 20 to 40 hours after incubation. According to Chen et al. (2014), fresh materials have faster mineralization rates and release more CO<sub>2</sub>. *G. sepium* treatment was observed with consistent high amounts of CO<sub>2</sub> released, followed by Azolla and chicken manure.

Haney et al. (2008) also found a strong relationship between organic carbon sources and CO<sub>2</sub> release in the first day of incubation. In their study on the effect of drying and rewetting, the greatest CO<sub>2</sub> respiration lasted for three days. The reason which led to this highest rate of CO<sub>2</sub> release might be due to the active microbial biomass breaking it down when they were exposed to dryness.

The incubation experiment also examined the mineralization rate of different organic materials over an 8-week period. The results showed that there were significant differences between treatments. The highest amount of CO<sub>2</sub> released in the first week was measured from *G. sepium* + RR with 375.13 mg kg<sup>-1</sup> soil. It was followed by Azolla and GS with 312.84 and 312.40 mg kg<sup>-1</sup> soil, respectively. Notably, their values are almost equal. Azola + SNAP and CM + SNAP rank next with 299.72 and 288.14 mg kg<sup>-1</sup> soil, respectively. There is a significant difference of the amount of CO<sub>2</sub> released from *G. sepium* compared the succeeding treatments ranked. In a similar study, Pangga et al. (2000) observed that more than 50% of total C has released during the first week of incubation.

A sharp decline in the amounts of CO<sub>2</sub> released was observed in Week 2. The highest amount of CO<sub>2</sub> released during this week was from CM and GS + RR, both yielding 93.28 mg kg<sup>-1</sup> soil. This was followed by Azola and CM + RR with close values of 83.60 and 80.96 mg kg<sup>-1</sup> soil, respectively. Differences of the CO<sub>2</sub> released among treatments were found to be insignificant since the measured CO<sub>2</sub> from these four treatments were ranked closely, From Week 2 to Week 6, CM and GS + RR recorded high amounts of CO<sub>2</sub> released compared to the other treatments. However, the highest amount of CO<sub>2</sub> released during Week 7 and 8 was measured from *G. sepium*.



**Fig. 2 Weekly carbon dioxide (mg kg<sup>-1</sup>) released over the period of eight weeks from different substrates and with the addition of SNAP solution**

Throughout the succession of weeks, the amounts of CO<sub>2</sub> lowered, although several peaks occurred, yet they were lower after each time. In the third week, there was an increased observation in CM+ SNAP treatment which measured 80.96 to 100.03 mg kg<sup>-1</sup>. Then there was another peak in AZ+ SNAP (60.72 to 65.41 mg kg<sup>-1</sup>). On the seventh week, there were three rises of CO<sub>2</sub> in GS, AZ and SNAP treatments (45.17 to 58.66, 38.72 to 55.78 and 41.65 to 45.32 mg kg<sup>-1</sup>, respectively). Makinde and Ayeni, (2013) also observed several peaks of CO<sub>2</sub> release in their study. The decomposition of OM can be divided into three stages—(i) active (1-6 weeks), (ii) reduced or slow (7-8 weeks) and (iii) stable or moderately

stable (9-16 weeks) (Ayeni, 2011). In the active stage, the most readily available and easily decomposed substrates and OM will be decomposed. At the same time, microorganism populations also increase quickly. When substrate quality was reduced, microorganism population becomes stable or dead and decomposed to serve as the nutrient source for other microorganisms and plants.

Cumulative CO<sub>2</sub> release also showed that addition of substrates increased the amount of CO<sub>2</sub> as compared with the treatments with substrates only (Figs. 1 and 2). Fresh materials have faster mineralization rates (Chen et al., 2014), especially *G. sepium* which consistent high amounts of CO<sub>2</sub> released was observed, followed by Azolla and chicken manure. Additional nutrient sources will increase CO<sub>2</sub> and CH<sub>4</sub> production (Gogo and Pearce, 2009). Generally low-quality soil carbon limits microorganism activities as well as carbon mineralization. When additional nutrient sources are added to the soil, microorganisms will attack these sources and rapidly increase their population (Fontaine et al., 2003).

## CONCLUSION

Mineralization of organic materials was about fifty percent (50%) in all substrates as compared to the amount of carbon dioxide released during the first week. The highest total CO<sub>2</sub> released was produced during the first week and the peak was between the first two days. The amount of CO<sub>2</sub> released less and less through time as the quality of the substrates eventually decreased. Using additional nutrient source (SNAP) to all organic materials resulted in faster rates of mineralization. Overall, the GS + SNAP treatment has the fastest rate of CO<sub>2</sub> release.

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## REFERENCES

- Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta A.M. and Tran, T.M. 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology & Biochemistry*, 41, 293-302.
- Ayeni, L.S. 2011. Cumulative effect of combined cocoa pod ash, poultry manure, NPK 20:10:10 fertilizer on major cations release for crop production in southwestern Nigeria. *Int. J. Agric. Soil Sci.*, 1 (7), 248-253.
- Bordilio, L.J., Singh, A.K., Patiram, M.K. and Hazarika, S. 2013. Microbial biomass nitrogen as an index of N availability in acidic soils of North East India. *Indian Journal of Hill Farming*, 26 (1), 22-28.
- Chen, X., Wang, X., Lieman, M., Cavigelli, M. and Wande, M. 2014. Influence of residue and nitrogen fertilizer additions on carbon mineralization in soils with different texture and cropping histories. *PLoS ONE* 9 (7): e103720. doi:10.1371/journal.pone.0103720.
- Chowdhury, N., Marschner, P. and Burns R. 2011. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. *Plant Soil*, 344, 241-254.
- Coûteaux, M.M., Bottner, P. and Berg, B. 1995. Litter decomposition, climate and litter quality. *Tree*, 10 (2), 63-66.
- Crohon, D. 2004. Nitrogen mineralization and its importance in organic waste recycling. UC Cooperative Extension, University of California, Davis, USA.
- Curtin, D. and Smillie, G.W. 1979. Original of the pH-dependent cation exchange capacities of Irish soil clays. *Geoderma*, 22, 213-224. Elsevier Scientific Publishing Company, Amsterdam.
- Deenik, J. 2006. Nitrogen mineralization potential in important agricultural soils of Hawaii. *Soil and Crop Management*, 15, 5.

- Eiland, F., Klamer, M., Lind, M.A., Leth, M. and Baath E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microb. Ecol*, 41, 272-280.
- Fontaine, S., Mariotti, A. and Abbadie, L. 2003. The priming effect of organic matter: A question of microbial competition? *Soil Biology & Biochemistry*, 35, 837–843. doi:10.1016/S0038-0717(03)00123-8
- Frankel, R.B. and Bazylinski, D.A. 2003. Biologically induced mineralization by bacteria. *Bio-mineralization*, 54, 95-114. ISSN 1529-6466, doi: 10.2113/0540095
- Franzluebbers, K., Weaver, R.W., Juo A.S.R. and Luebbers, A.J. 1994. Carbon and nitrogen mineralization from cowpea plants part decomposing in moist and in repeatedly dried and wetted soil. *Soil Biology and Biochemistry*, 26, 1379-1387.
- Gaskell, M. and Smith, R. 2007. Nitrogen sources for organic vegetable crops. *Horttechnology*, 17 (4), 431-441.
- Gogo, S. and Pearce, D.M.E. 2009. Carbon, cations and CEC: Interactions and effects on microbial activity in peat. *Geoderma*, 153, 76-86.
- Haney., R.L. Brinton, W.H. and Evans, E. 2008. Estimation soil carbon, nitrogen, and phosphorus mineralization from short-term carbon dioxide respiration. *Communications in Soil Science and Plant Analysis*, 39, 2706-2720. ISSN 0010-3624 print/1532-2416 online, doi: 10.1080/00103620802358862
- Hartz, T.K., Mitchell, J.P. and Giannini, C. 2000. Nitrogen and carbon mineralization dynamics of manures and composts. *HORTSCIENCE*, 35 (2), 209-212.
- Hartz, T.K. and Brittan, K. 2003. Nitrogen mineralization rate of biosolids and biosolids compost. Department of Vegetable Crops, University of California, Davis, USA.
- Howard, D.M. and Howard, P.J.A. 1993. Relationships between CO<sub>2</sub> evolution, moisture content and temperature for a range of soil types. *Soil Biology and Biochemistry*, 25, 1537-1546.
- Keift, T.L., Soroker, E. and Firestone, M.K. 1987. Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology and Biochemistry*, 19, 119-126.
- Li, S.T. and Li, L.L. 2014. Nitrogen mineralization from animal manures and its relation to organic N fractions. *Journal of Integrative Agriculture*, Advance Online Publication. doi: 10.1016/S2095-3119(14)60769-3.
- Makinde, E.A. and Ayeni, L.S. 2013. Determination of mineralization rate of organic materials using carbon dioxide evolution as an index. *International Journal of Plant & Soil Science*, 2 (1), 16-23.
- Ouyang, X.J., Zhou, G.Y., Huang, Z.L., Liu, J.X., Zhang, D.Q. and Li J. 2008. Effect of simulated acid rain on potential carbon and nitrogen mineralization in forest soils. *Pedosphere* 18 (4), 503-514.
- Pangga, G.V., Blair, G. and Lefroy, R. 2000. Measurement of decomposition and associated nutrient release from straw (*Oryza sativa* L.) of different rice varieties using a perfusion system. *Plant and Soil*, 223, 1-11.
- Pereira, J.L., Trindade, H. and Moreira, N. 2006. Nitrogen mineralization in soils receiving different rates of cattle-slurry and cropped with forage maize. *Grassland Science in Europe*, 11, 724-726.
- Schrama, M., Veen, G.F., Bakker, E.S., Ruifrok, J.L., Bakker, J.P. and Olf, H. 2013. An integrated perspective to explain nitrogen mineralization in grazed ecosystems. *Perspectives in Plant Ecology, Evolution and Systematics*, 15, 32-44.