Mineralization dynamics of different commercial organic fertilizers from agro-industry organic waste recycling: an incubation experiment

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ABSTRACT

The mineralization process of different commercial organic fertilizers was investigated in controlled laboratory conditions. The soil was mixed with the following organic fertilizers: Emos CAP®️, Organ CAP®, Sic Stal®️ and urea (as a control) at the rate of 300 mg N/kg dry soil. Emos CAP®️ is made of cattle and poultry manure, meat, bone meal and dried blood, Organ CAP®️ is a product made of leather and skins, while Sic Stal®️ contains cow and horse manure. During the incubation the concentration of NO\textsubscript{3}\textsuperscript{–}-N, NH\textsubscript{4}\textsuperscript{+}-N, microbial biomass and carbon dioxide, nitrous oxide and ammonia emissions were determined. All fertilizers showed a peak of NH\textsubscript{4}\textsuperscript{+}-N after 7 days from the beginning of the test. The decomposition of Sic Stal®️ caused a rapid rise of CO\textsubscript{2} production associated to the growth of microbial biomass while Emos CAP®️ promoted a release of N\textsubscript{2}O in the first 16 days. In conclusion, all the commercial organic fertilizers tested can be considered fertilizers with a fast release of N, among them Emos CAP®️ and Sic Stal®️ allow a rapid N supply to plants while Organ CAP®️ could be used when the N request of plants is not immediate.

Keywords: NO\textsubscript{3}\textsuperscript{–}-N; NH\textsubscript{4}\textsuperscript{+}-N; microbial biomass; N\textsubscript{2}O; CO\textsubscript{2}; NH\textsubscript{3}

Global mineral nitrogen (N) fertilizer application rate increased almost 20 fold in the last 50 years, reaching almost 100 million t/year; but an important portion of this is lost under field conditions (Glass 2003). The risk of N leaching or emission in the atmosphere as long as the low level of organic matter (OM) in most agricultural soil, has led to an increased interest on sustainable agricultural practise such as the use of organic matter for plant nutritional management. In sustainable orchard management, the use of organic material such as crop residues, animal manure, compost, agro-industry or animal by-products is encouraged as they are essential to restore or maintain soil chemical, physical and biological properties, optimal for crop growth (Diacono and Montemurro 2010). Organic fertilizers represent a source of nutrient for plants (Powlson et al. 2011) since their decomposition is responsible for nutrient release; moreover, animal by-products are rich in protein and therefore they offer a good source of N for plant (Gaskell and Smith 2007). As a consequence of soil application, OM is mineralized by soil microorganisms; inorganic ions are released as a result of this process and can be absorbed by plants, adsorbed by soil colloids or leached through the soil profiles into aquifers. The evolution of OM in soils depends on many factors such as temperature, humidity, soil texture, agronomic practices (Pierzinski et al. 2000) and the nature of the organic matrix applied (Swift et al. 1979).

The mineralization process of organic material should be investigated in order to optimize the induced soil nutrient availability to tree demand. The aim of this investigation was to evaluate, in laboratory conditions, the different mineralization rates of some commercial organic fertilizers obtained from recycling of agro-industry waste and suitable for low input agricultural management.

MATERIAL AND METHODS

A clay loam Bathicalci Eutri Cambisol soil (FAO 2010) was collected from the field of the
Experimental station of the University of Bologna, in Cadriano (44°35’N, 11°27’E), mixed with sand at a ratio soil: sand of 60:40 to reduce the presence of clay, sieved to 2 mm and incubated at 20°C at constant soil humidity (equal to field capacity) for 10 days before use. The soil was characterized by 23% sand, 49% silt, 23% clay, 1.4% of OM, 1.1% of total N and pH 7.5. Soil was then mixed with commercial organic fertilizers as: Emos CAP®, Organ CAP®, Sic Stal® and urea as control at the rate of 300 mg N/kg dry soil. Also an unamended soil was introduced as unfertilized control in the experiment. Emos CAP® (8% N; 31–34% C) is obtained from cattle manure, poultry meat and bone meal and dried blood, Organ CAP® (11% N; 40% C) is a product made of leather and skins, while Sic Stal® (3% N; 28% C) contains cow and horse manure.

For each treatment, soil was placed in 1 kg plastic pot (4 pots/treatment) and a sub sample for each replicates of 50 g was put in 250 mL glass jar (4 jars/treatment); covered with a perforated black polyethylene bag to allow aeration and incubated at constant air temperature of 22.5 ± 1.2°C and relative air humidity of 57.9 ± 2.3%. Throughout the incubation period soil moisture was maintained at field capacity by adding distilled water when necessary. After 2 and 24 h, 1 week, 1, 3 and 5 months, the soil of the pots was sampled to perform analysis of the concentration of NO$_3^-$-N, NH$_4^+$-N and microbial biomass. Nitrate- and ammonium-N were extracted from 10 g of soil by a solution of 100 mL of KCl (2 mol/L); samples were shaken at 110 rpm for 1 h and after soil sedimentation, limpid solution was collected and stored at −20°C until analysis, made with an auto analyzer (Auto Analyzer AA-3; Bran + Luebbe, Norderstadt, Germany). Microbial biomass carbon (C) was measured using the substrate induced respiration (SIR) method (Anderson and Domsch 1978): 50 g of fresh soil were mixed with 200 mg of glucose and incubated at 24°C for 4 h. The accumulation of carbon dioxide (CO$_2$) was measured with a photo-acoustic infrared gas analyzer (Inova 1302, LumaSENse Technologies A/S, Ballerup, Denmark); and was converted into microbial C according to Anderson and Domsch (1978).

In the same days of nitrate and ammonium analysis, soil pH was determined on 10 g of dry soil mixed with 25 mL of distilled water; the solution was shaken at 110 rpm for 2 h, left to stand (Violante and Adamo 2000) and then pH was measured by pH-meter electrode (Basic 20, Crison, Crison instruments, Barcelona, Spain).

In addition, each day in the first week, and then two/three times a week, for a total of 19 analysis over the first 3 months CO$_2$, nitrous oxide (N$_2$O) and ammonia (NH$_3$) fluxes were measured with a photo-acoustic infrared gas analyzer (Inova 1302, LumaSENSE Technologies A/S, Ballerup, Danmark). Ammonia determinations started seven days after the beginning of the experiment. Jars were sealed for 3 h; once CO$_2$ and NH$_3$ fluxes were determined, jars were opened, aerated and sealed again for 3 h; a soda lime filter was used to minimize CO$_2$ interference during N$_2$O flux measurement (Velthof et al. 2002). Four empty jars were used to correct values for background CO$_2$, NH$_3$ and N$_2$O concentrations.

The net mineralization was calculated as the difference between the amount of mineral N in the soil (NO$_3^-$ + NH$_4^+$) in the amended and in the unamended soils and was expressed as % of the N applied at the beginning of the experiment.

Statistical analysis. Data were statistically analyzed as in a factorial experimental design with soil fertilization and time as main factors. When analysis of variance showed a statistical effect of treatments ($P \leq 0.05$), means were separated by Student Newman Keuls test; when interaction between factors was significant, 2 times standard error of means (SEM) was used as the minimum difference between two means statistically different at $P \leq 0.05$.

RESULTS

Two hours after the treatments the availability of NH$_4^+$-N was increased by the application of Emos CAP® and Sic Stal® compared to the other treatments that were not different from the untreated control (Figure 1). Twenty-four hours after the beginning of the incubation the application of urea increased the availability of NH$_4^+$-N that was similar to Sic Stal® but still lower than Emos CAP®. After 1 week, the soil treated with urea presented the highest NH$_4^+$-N values followed by the application of Emos CAP®; Organ CAP® showed values higher than control and not different from Sic Stal® (Figure 1). One month after the beginning of the experiment the availability of NH$_4^+$-N decreased if compared with the values measured after one week and the differences among treatments disappeared. Three months after the application of fertilizers, NH$_4^+$-N soil concentra-
tions were similar for all treatments and lower than the previous determinations (Figure 1). At the end of the experiment all treated pots showed a slight increase of NH$_4^+$-N concentration even if not significantly different from control (Figure 1).

Soil concentration of NO$_3^-$-N increased 1 week after the application of fertilizers if compared with unfertilized soil, with no differences among treatments. One month after fertilization, soil NO$_3^-$-N concentration was greater for urea, followed by Emos CAP® and Organ CAP® (Figure 2). The availability of NO$_3^-$-N, at the end of the experiment, was increased by urea and Emos CAP® compared to Organ CAP® that was higher than Sic Stal®, and all treatments were higher than unfertilized control (Figure 2).

Sic Stal® application determined an increase of microbial biomass during the entire incubation period that was observed first 24 h after the beginning of the trial, when Sic Stal® showed values higher than the other treatments (Figure 3). The highest values were observed one week from fertilizers application, while the lowest values were those detected 5 months after the application of fertilizers when all treatments showed similar values and higher than untreated control (Figure 3). After one week also Emos CAP® showed a peak of microbial activity that was lower than Sic Stal® but higher than all other treatments (Figure 3). One month after treatment application, microbial C values decreased and remained stable until the end of the experiment (Figure 3).
Two and 24 h after treatment application, soil fertilized with urea showed the highest pH, followed by the untreated control, while Emos CAP® and Sic Stal® showed similar values, but lower than urea and control (Figure 4). Organ CAP® had intermediate values similar to both control and other organic fertilizers (Figure 4). Seven days after treatment, the application of urea showed a decrease of soil pH if compared with the values measured at 2 and 24 h; Sic Stal® showed the lowest values, while Emos CAP® and Organ CAP® had values similar to the previous measurements and other treatments. A decline occurred for all treatments after 1 week and 1 month (Figure 4), while at the end of the incubation there was a slight increase of values (Figure 4).

There was an increase in CO₂ emission in the first 20 days of incubation, then the production levelled off for all treatments with the exception of Sic Stal® that showed a constant increase of CO₂ all over the incubation (Figure 5). Sic Stal® always showed the highest CO₂ emission values followed by Emos CAP®, Organ CAP® and urea; control soil showed the lowest emission (Figure 5). The N₂O cumulative fluxes after Emos CAP® application sharply increased since day 16, then it became linear and remained always higher than all other treatments (Figure 6). Urea and Organ CAP® also showed an increase of N₂O flux lower than Emos CAP®, but higher than Sic Stal® and control, the emission increased until day 28 and then remained stable (Figure 6). The amendment with Sic Stal® determined an increase of N₂O production if compared to control soil, but lower that all other fertilization treatments and linear starting from day 16 (Figure 6).

Urea soils showed a rapid NH₃ emission in the first 16 days of incubation and then it stabilized around 200 µg/h/kg (Figure 7); also control had a sharp emission of this gas that increased over time.
and became higher than urea starting from day 52 (Figure 7). Organic fertilizers always maintained the lowest values and similar between each other (Figure 7).

During the whole incubation period the net mineralization increased from 9.6% (2 h after treatments) to 55.1% (6 months). Urea showed values (50.2%) not different from Emos CAP® (42.2%) but higher than Organ CAP® (32.6%) and Sic Stal® (24.4%); Emos CAP® was similar to Organ CAP® but significantly higher than Sic Stal®; Organ CAP® and Sic Stal® were not statistically different.

**DISCUSSIONS**

The release of N and other nutrients in the soil depends on chemical characteristics of organic material and it is influenced by a wide range of factors such as the C:N ratio, N and lignin content, C concentrations, polyphenol:N and polyphenol plus lignin:N ratios (Bending et al. 1998 and literature here cited). In general, matrices with a C:N ratio > 30 lead to a sharp increase in the microbial activity and a sequestration of N in the soil (Pierzinski et al. 2000). On the other hand, when the matrix has a low (between 3.6 to 9.3) C:N ratio, high quantities of NO$_3^-$-N are released with possible loss in the environment (Pierzinski et al. 2000). Organic fertilizers used in this incubation experiment were characterized by low C:N ratio and mineralization occurred at a different rate; the release of NO$_3^-$-N by Sic Stal® (the matrix with the highest C:N ratio = 9.34) was slower compared to other fertilizers. Moreover, at the end of the experiment, the N mineralized by Sic Stal® was lower (33%) than

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**Figure 5.** Effect of fertilization treatments on CO$_2$ flux as observed during the 5 months of mineralization assay. Interaction between treatment and time significant at $P \leq 0.001$. Values differing by 2 standard error of means (SEM) are statistically different. DW – dry weight

**Figure 6.** Effect of fertilization treatments on N$_2$O flux as observed during the 5 months of mineralization assay. Interaction between treatment and time significant at $P \leq 0.001$. Values differing by 2 standard error of means (SEM) are statistically different. DW – dry weight
other treatments (urea 70%, Organ CAP® 66% and Emos CAP® 51%).

All fertilizers showed a 7 day period of immobilization in the soil, followed by a peak of the release of NH₄⁺-N. The prompt urea hydrolysis resulted in an accumulation of NH₄⁺-N in the first days after its addition and a release of NH₃; the NH₄⁺-N produced was then in part oxidised to NO₃⁻-N in about 1 month after application of the fertilizer. The peak of NH₄⁺-N, 7 days after the addition of urea is reported in literature (Sahrawat 1980), and is the result of urea hydrolyzation from urease responsible also for the rise in pH. The sharp decrease of NH₃ is the result of volatilization and the concomitant oxidation of NH₄⁺ to NO₃⁻. The behaviour of Emos CAP® was similar to urea because of the presence of blood meal that is mainly composed of globular proteins that are fast mineralized in soil (Cayuela et al. 2009, Sinicco et al. 2010). Emos CAP® however, showed values lower than urea probably due to the presence of manure, meat and bone meal that have a rate of mineralization lower than blood. This was evident and proved by the net mineralization rate that was similar between urea and Emos CAP®. Organ CAP® is composed of hydrolyzed leather characterized by partially hydrolyzation of protein (Barajas-Aceves and Dendooven 2001) having an intermediate degree of degradability if compared to blood or manure as was also demonstrated by the intermediate values of net N mineralization.

Soil CO₂ flux is the result of decomposition of OM by soil microorganism; unlike urea, all organic fertilizers significantly increased CO₂ flux for a short time after application. In particular Sic Stal® caused, in the initial stages, a rapid rise in CO₂ production associated to the growth of microbial biomass stimulated by the fraction of manure that is included in the fertilizers, which is in line with the results reported in literature (Heinze et al. 2010). Other studies (Jannoura et al. 2013) have demonstrated a highest production of CO₂ after horse manure application, if compared to compost. Several authors (Flessa and Beese 2000, Van Groeningen et al. 2004) reported an increased N₂O emission after application of organic matter; in particular, easily available organic matter fractions were found to promote N₂O emissions since they provide an easily available substrate for nitrification and denitrification (Velthof et al. 2003).

The different gas emission dynamics between treatments are probably related to different chemical complexity of the protein structure of the fertilizers used (Sinicco et al. 2010) and to differences in the nature of C and N components present in each fertilizer. Manure contains high amounts of cellulose and hemicellulose, low amounts of lignin; hence, it may be hypothesized that these components have a more rapid mineralization than other matrix. On the contrary, in other fertilizers used such labile compounds were present in lower proportions and gas emissions were lower.

In conclusion, the experiment showed how a proper mixture of matrices with different origin may allow obtaining fertilizers with a different release of N suitable for different agronomic conditions and plant requirements. The commercial organic fertilizers tested showed a fast release of N, among them Emos CAP® had a rapid N supply to plants similar to urea, while Organ CAP® and Sic...
Stal® could be used when the N request of plants is not immediate. However, care should be paid when transferring incubation experiment data in field since temperature, soil moisture and agro-nomic practises strongly influence decomposition.

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