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Short-term changes of soil microbial functional diversity induced by mineral and organic fertilizers

Schimbări de scurtă durată a diversității funcționale a comunității microbiene din sol datorate fertilizării minerale și organice

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ABSTRACT

The use of both mineral and organic fertilizers is a key agricultural practice with a relevant impact on soil processes and fertility. This study examined how three organic fertilizers (slurry, cattle manure and mustard as green manure) and one chemical fertilizer (ammonium nitrate) can affect soil microbial functional diversity and soil biochemical processes. A short-term experiment was organized under controlled conditions for 30 days. Soil samples were collected every ten days in order to measure soil pH, N-NH_4, N-NO_3 and microbial activity as a community-level physiological profile (CLPP). The MicroResp method was used to assess community-level physiological profiles by using 15 different carbon sources and converting their catabolic activity to CO_2. The obtained results showed that cattle manure and slurry have a significant impact on CLPP and mineralization during the first 10 days while mustard applied as green manure impacted soil parameters after 10 days. The use of chemical fertilizer had limited effect on CLPP. The organic substrates α-ketoglutaric acid and oxalic acid gave the highest metabolic activity and drove the pattern use substrate in the experimental soils.

Keywords: community-level physiological profiling, MicroResp, substrate utilization, microbial community, fertilization

REZUMAT

Utilizarea fertilizanților minerali și organici în agricultură este o practică cu impact semnificativ asupra proceselor din sol și a fertilității acestuia. Studiul prezentat evaluează efectele a trei ferilizanți organici (dejecțiile lichide, gunoiul de grajd, muștarul ca îngrășământ verde) și a unui fertilizant chimic (azotatul de amoniu) asupra diversității funcționale a comunității microbiene a solului și a proceselor biochimice din sol. Un experiment de scurtă durată a fost organizat în condiții controlate pentru o perioadă de 30 de zile. La intervale de 10 zile s-au colectat probe de sol pentru cuantificarea unor caracteristici chimice ale solului și a activității metabolice a comunității microbiene din sol. Metoda MicroResp a fost folosită pentru realizarea profilului fiziologic al comunității microbiene folosind 15 surse de carbon diferite oferite ca substrat metabolic. Rezultatele obținute au arătat că gunoiul de grajd și dejecțiile lichide au un impact semnificativ asupra CLPP și a mineralizării în primele zile după aplicare, în timp ce muștarul integrat în sol afectează semnificativ procesele biochimice după 10 zile de la aplicare. Folosirea fertilizanților chimici au un impact limitat asupra profilului fiziologic al comunității microbiene. Acidul α-ketoglutaric și acidul oxalic au fost substraturile organice cel mai intens utilizate de către comunitatea microbiană din sol.

Cuvinte cheie: profilul fiziologic al comunității microbiene, metoda MicroResp, substraturi utilizate, comunitate microbiană, fertilizare

INTRODUCTION

Nowadays high amounts of mineral and organic fertilizers are used in agricultural soils to supplement or improve the soil capacity to provide nutrients for crop production (Barabasz et al., 2002). It is considered that more than half of the world crop yield is attributed to fertilizers (Stewart et al., 2005) with a total world commercial fertilizers use of 179 million metric tonnes of nutrients (Singh and Ryan, 2015). Besides the benefits of using fertilizers, much consideration is given to the possible negative effects of their use. Soil organic matter loss (Herencia et al., 2007; Iovieno et al., 2009), water pollution (Moss, 2008), yield quality (Schipanski et al., 2014) and soil biodiversity loss (Singh and Ryan, 2015;) are the most often discussed issues related to the misuse of the fertilizers. The new approach to agricultural production is based on more agroecological principles which are able to provide solutions for the reduction of the environmental negative impact of agriculture. Related to fertilizers, the need for sustainable management without compromising soil health is generally accepted. The improvement of fertilizer use efficiency is a goal for both researchers and farmers and soil biota should be considered as a key factor in assurance of best fertilizer practices. The use of organic fertilizers and the soil practices that bring organic matter into the soil are considered beneficial for soil health due to the improvement of biological processes in the soil. When organic fertilizers are applied to the soil, the structure of microbial communities and their metabolic capabilities will assure the availability of nutrients to the plants via the mineralization process. Kuzyakov et al. (2000), described as priming effect of the changes in soil organic matter decomposition after the addition of organic or mineral substances to the soil. Blagodatskaya and Kuzyakov (2008) discuss about apparent priming effect which is an activation of microbial metabolism not related to the SOM turnover. When new organic matter is added to the soil, we can expect an activation of dormant biomass which becomes metabolically active and starts to use different organic substrates. The mineralization process is accelerated and changes in CO_2 efflux and nitrogen mineralization rate happen. For a period of time, the highest amount of nutrients will be released consistent with the quality of organic matter and climatic conditions. Different authors reported that the effect can last between 3 days (Blagodatskaya and Kuzyakov, 2008) to more than 2 months (Masunga et al., 2016), related to the quality of organic matter added to soil.

Soil microbial metabolic activity is the driving force of the soil organic matter mineralization process through which nutrients are released into the soil (Grayston et al., 2004; Esperschütz et al., 2007). Community-level physiological profile (CLPP) is a soil biological parameter which can be used to assess short-term changes in functional diversity and activity of microbial community (Frąc et al., 2012; Gartzia-Bengoetxea et al., 2016). CLPP describes the ability of soil microorganisms to catabolize a range of different organic substrates and it can provide a better understanding of microbial roles in the ecosystem (Lalor et al., 2007; Pignataro et al., 2012; Sradnick et al., 2013). CLPP can express the functional diversity of the soil microbial community and its potential metabolic activity. One method used to assess microbial functional diversity is the MicroResp method (Campbell et al., 2003), which measures the CO_2 produced by the microbial community during the mineralization of easily available carbon sources which are similar to root exudates and other metabolites present in the soil. The method uses 15 carbon sources containing carbohydrates, amino acids, amino sugars and carboxylic acids, which are usually dissolvable in water and are selected to be ecologically relevant (Chapman et al., 2007). The selected substrates are organic compounds present in root exudates or formed during litter decomposition. Their presence can affect soil chemistry, soil microbial diversity and metabolic activity (Orwin, 2006). Development of agricultural management strategies which rely on ecosystem self-regulation by manipulating soil microbial biota to increase soil biological processes, presents more interest (Bardgett and McAlister, 1999). In this context, it is essential to provide more direct insights into the changes in microbial diversity and activity during the priming effect process. How different kinds of organic matter will affect the diversity and activity of the soil microbial community

and how these changes will influence the soil nutrient availability are unknown aspects related to organic and mineral fertilization of agricultural soils.

The present study aimed to investigate the effect of chemical and organic fertilizers on microbial metabolic activity and functional diversity in a short-term experiment. The dynamic of nitrogen mineralization in relation to microbial metabolic activity was assessed in a short time period in order to predict nutrient availability for the plant after fertilizers addition to the soil.

MATERIALS AND METHODS

Experimental design and soil proprieties

A microcosm experiment was conducted under controlled temperature and humidity conditions by using Phaeozem soil (clay 44%, silt 28%, and 28%) collected from an arable field where conventional agriculture was practised for a long time. Soil pH at the start of the experiment was neutral (pH - 7.22), while $N\text{-}NO_3$ and $N\text{-}NH_4$ registered 24.5 mg/kg soil, respectively 6.7 mg/kg soil. Available phosphorus at the start of the experiment recorded 24.3 mg P_2O_5/100 g soil, while the amount of available potassium was 66.4 mg K_2O /100 g soil. The soil was sieved through a 2 mm mesh to remove the large organic matter and stones. Fifteen pots 25 cm in height and 6.6 cm in diameter were filled with 2500 cm^3 soil to a bulk density of 1.2 g/cm^3. The water content of the soil was adjusted to 20% (200 g water for 1000 g dry soil) and 4 different fertilizers were added to the experimental pots: cattle slurry (CS), cattle manure (CM), mustard as green manure (IV) were used as organic fertilizers while ammonium nitrate was used as mineral fertilizer (M). The fertilizers were mixed with the first 10 cm of the soil and the application rate was calculated at 150 kg N/ha for all treatments (Table 1). Control treatment (Co) did not receive any fertilizers. All treatments were made in triplicate (n = 3) and the experimental pots were covered with 20µm fine mesh and maintained at 20 °C and 20% water content for 30 days. This way the experiment simulates a period when the root system is not present and has limited influence on soil processes.

Soil sampling and chemical analysis

Soil samples were collected from each pot after 10, 20 and 30 days of incubation. A small soil corer was used to extract 150 g of soil from each experimental pot. Extracted soil was sieved through a 2 cm mesh sieve and only soil passing the sieve was used for the analysis. The material retained by the sieve was put back into the pot. After extraction in each pot, the surface soil was gently arranged and wetted. Extracted soil was used to measure pH, $N\text{-}NO_3$, N-NO4 and CLPP at each sampling time. Organic carbon, total nitrogen, available phosphate and available potassium were measured at the beginning and end of the experiment. Soil pH was measured electrochemically in water (1 to 5 ratio), using a Consort C863 multianalyser. $N\text{-}NO_3$ and $N\text{-}NH_4$ were measured with an ion-selective electrode after extraction with $CaCl_2$. Available phosphorus and potassium were measured colorimetrically after extraction with ammonium lactate and C_{org} and N_t were measured by dry combustion with LECO TruSpec CN.

Community-level physiological profiling

MicroResp multi-SIR method was used to measure the soil microbial physiological profiles. MicroResp gives a quick response overview of microbial activity and diversity by measuring responses after 6 hours of incubation (Campbell et al., 2003). The soil samples were adjusted to 45% of their water-holding capacity (WHC) and 35 g of soil was added to 96 deep-well plates in equal quantities

Table 1. Soil and fertilizers' chemical characteristics at the start of the experiment

Parameters	Soil	Slurry (CS)	Manure (CM)	Mustard (IV)	Mineral (M)
C_{org} (%)	2.2	41.9	35.7	40.7	-
N_t (%)	0.19	2.36	3.36	4.16	33.50
C/N	11.5:1	18.2	10.6	9.7	-

by using a specific device. Then 25 µl of fifteen different carbon source solutions and distilled water were added to the soil by using a multichannel pipette. The carbon sources used were deionized water (basal respiration), 4 carboxylic acids (α-ketoglutaric acid, L-malic acid, citric acid, oxalic acid), 5 carbohydrates (D-galactose, L-arabinose, D-fructose, D-glucose, D-trehalose), 5 aminoacids (γ-aminobutyric acid, L-alanine, L-arginine, L-lysine, L-cysteine) and 1 aminosugar (N-acetyl-glucosamine). Carbon source aqueous solutions were prepared at 30 mg g^{-1} H_2O concentration. The plates were kept open for 30 minutes to avoid considering abiotic CO_2 production in the final calculation (Creamer et al., 2016). After 30 minutes the detection plates were fixed on the top of the deep plates and CO_2 produced in each well was colorimetrically quantified. The colour development in the detection plate was measured before and after six hours of incubation at 25 °C by using a Biotek Epoch Multipoint Spectrophotometer at 570 nm wavelength. The detection plate is a system that detects the released carbon dioxide using cresol as an indicator (Campbell et al., 2010). C-CO_2 production rate was calculated with a formula that converts normalized absorbance data (Ai) to %CO_2: %CO_2 = A + B / (1 + D x Ai), where A = -0.2265, B = -1.606, D = - 6.771 (Campbell et al., 2010).

Statistical analysis

The results presented in the table are mean values and standard error for each treatment and sampling time (n = 3). ANOVA was used to analyze the effect of fertilizer type and sampling time on soil parameters. Data analysis was performed with RStudio (R Core Team. 2024.), version 2024.04.0+735. Basic statistics were extracted with “psych” package formulas (Revelle, 2024), from which means and standard errors were extracted. The differences between fertilizers treatments and sampling dates were assessed with the post-hoc LSD test (at $P < 0.05$), with formulas from “agricolae” package (de Mendiburu, 2023). Pearson correlations were calculated to show relationships between soil parameters and specific metabolic activity in different fertilized soils and at different times. Results were presented as correlograms designed with a "corrplot“ package (Wei and Simko, 2021).

RESULTS AND DISCUSSION

Soil chemical proprieties

The type of fertilizers and sampling date affected significantly all the soil parameters. The most important factor was sampling time (F = 30,25, $P < 0,01$), followed by type of fertilizers (F = 9.3, $P < 0,01$) and combination of both factors (F = 4.1, $P < 0,01$).

The presence of organic and mineral fertilizers in the soil during the first 10 days of incubation led to a significant increase of N-NO_3 in all treatments (Table 2). The highest value was reported in manure treatment (201 mg/kg soil), while the lowest increase was recorded for control (55.12 mg/kg soil). The increase of soil N-NO_3 was associated with a decrease of soil N-NH_4 in all treatments where organic amendments were used. Only for mineral treatment, a significant increase of N-NH_4 was measured after the first 10 days of incubation reaching the value of 45.29 mg/kg soil.

After 20 days of incubation, N-NO_3 showed the highest values for most of the treatments. The high N-NO_3 production rate was more evident for green manure treatment (149.1 mg/kg soil) and reached the maximum value for mineral treatment (352.6 mg/kg) (Table 2). For slurry and control treatments the measured values were not significantly different from the values recorded at the first sampling time. Manure treatment showed a significant decrease of N-NO_3 compared with the first sampling date, but the recorded values were still high indicating an intense nitrification process in the soil. After 20 days of incubation, N-NH_4 showed the highest values measured in all treatments, excluding minerals, but the increase was not significantly different compared with other values (Table 2). For the mineral treatment soil, a significant decrease of N-NH_4 was reported compared to the previous measurement.

Table 2. Soil chemical parameters measured during the experiment

Treatment	Sampling	pH	$N\text{-}NO_3$ (mg/kg soil)	$N\text{-}NH_4$ (mg/kg soil)
Co	10 days	7.49 ± 0.04^{b}	55.12 ± 5.49^{fg}	0.32 ± 0.02^{b}
	20 days	7.18 ± 0.01^{d}	48.38 ± 6.86^{gh}	1.77 ± 0.18^{b}
	30 days	7.51 ± 0.02^{b}	13.90 ± 0.28^{i}	1.66 ± 0.30^{b}
CS	10 days	7.36 ± 0.04^{c}	56.12 ± 7.59^{fg}	0.61 ± 0.02^{b}
	20 days	7.26 ± 0.01^{cd}	52.13 ± 3.06^{fg}	3.25 ± 0.45^{b}
	30 days	7.55 ± 0.09^{b}	25.78 ± 4.38^{hi}	1.32 ± 0.44^{b}
CM	10 days	7.51 ± 0.05^{b}	201.1 ± 13.95^{b}	2.33 ± 0.52^{b}
	20 days	7.51 ± 0.04^{b}	58.50 ± 0.73^{efg}	4.97 ± 0.22^{b}
	30 days	7.80 ± 0.04^{a}	46.29 ± 4.21^{fg}	2.33 ± 0.63^{b}
IV	10 days	7.20 ± 0.02^{d}	73.22 ± 6.81^{def}	3.42 ± 0.24^{b}
	20 days	6.99 ± 0.04^{e}	149.12 ± 16.05^{c}	2.76 ± 0.04^{b}
	30 days	7.19 ± 0.07^{d}	60.76 ± 2.26^{efg}	1.56 ± 0.14^{b}
M	10 days	7.90 ± 0.03^{a}	80.65 ± 11.15^{de}	45.29 ± 9.52^{a}
	20 days	6.46 ± 0.01^{f}	352.67 ± 12.34^{a}	5.45 ± 1.28^{b}
	30 days	7.02 ± 0.06^{e}	95.74 ± 7.22^{d}	4.17 ± 1.10^{b}

Data are mean ± S.E. Values followed by the same letter are not significant ($P < 0.05$)

At the end of the experiment (after 30 days) a decrease of both $N\text{-}NO_3$ and $N\text{-}NH_4$ was reported for all treatments (Table 2). The decrease was more evident for $N\text{-}NO_3$ while $N\text{-}NH_4$ showed similar values with the second sampling date. Control treatment recorded the lowest concentration of soil $N\text{-}NO_3$ and $N\text{-}NH_4$ at each sampling date compared with other treatments. Short-term changes in nitrogen mineralization in soil treatments were affected by the type of fertilizers used and the time of sampling. The obtained results showed that nitrogen mineralization started immediately after fertilizer application and reached the highest value after 10 days for slurry and manure treatments and after 20 days for green manure treatments. Previous studies with different organic fertilizers applied to the soil have shown that nitrogen mineralization depends on the C: N ratio of the organic material (Sandor et al., 2011; Cordovil et al., 2005; Mohanty et al., 2011) and the metabolic activity of the microbial community (Manojlovic et al., 2010; Sradnick et al., 2013). Cattle manure and green manure used in our experiment had low values of C: N ratio (10.6, respectively 9.7) which conducted an intense nitrification process during the first days of incubation. The process was rapid for manure treatments during the first 10 days, while for green manure was more intense between days 10 and 20 of incubation. The presence of cellulose, hemicelluloses and other phenolic compounds in green manure can slow nitrogen mineralization (Calderón et al., 2005). That can explain the high nitrogen mineralization rate during the first 10 days of incubation in slurry and cattle manure treatments, while for green manure treatment, mineralization was more intense after 10 days. The decrease of $N\text{-}NO_3$ and $N\text{-}NH_4$ between day 20 and day 30 of the experiment resulted as a consequence of the reduction of easily mineralized organic N compounds that were rapidly mineralized during the first 20 days of incubation. Cordovil et al. (2005) reported similar results for composted pig manure and pointed out that N stable recalcitrant compounds resulted after 35 days of incubation.

We assume that the release of N-NO_3 and N-NH_4 in mineral treatment was mainly related to the solubilization process and not to microbial metabolic activity. The high solubility of ammonium nitrate resulted in a high release of N-NO_3 and N-NH_4 during the first 20 days of the experiment, followed by a reduction of soil mineral nitrogen during the last part of the experiment.

Soil pH changes measured during the experiment were related to the type of fertilizers and sampling date. The range of soil pH varied between 6.99 measured in green manure treatment and 7.90 measured in mineral treatment (Table 2). During incubation, an increase in soil pH was observed for cattle manure and slurry treatments while for green manure treatment, pH values did not change significantly. In mineral treatment, a significant increase was measured after 10 days of incubation followed by a significant decrease after 20 days and 30 days of incubation. It was suggested by others (Sradnick et al., 2013; Kemmit et al., 2006) that small changes in soil pH can affect mineralization and microbial metabolic activity of the soil. Contrary, Adams and Adams (1983) reported no effects of soil pH on basal respiration. In most of the cases, the reported values of pH in our experiment were close to the neutrality point (pH = 7) which has a limited impact on soil chemical and biological processes. Similar to the results presented by Curtin et al. (1998), we did not observe a direct effect of pH on mineralization and microbial activity in the experimental soils.

Soil microbial community physiological profile

A community-level physiological profile was used to assess changes in microbial metabolic activity in experimental treatments. Four groups of organic substrates were used for this assessment: carbohydrates, carboxylic acids, amino sugars and amino acids. C-CO_2 released from the soil without any added organic substrate was considered soil basal respiration. The total respiration rate, calculated as the mean respiration rate for all specific substrates and water, was higher for green manure treatments, followed by manure, slurry, control and mineral treatments (data not shown).

The addition of organic fertilizers boosted metabolic activity in all treatments, which resulted in a high rate of carbon and nitrogen mineralization. This result was visible after 10 days of incubation but reached the maximum level after 20 days of incubation (Table 3, 4, 5, 6). After 30 days of incubation metabolic activity decreased indicating that the peak of metabolic activity was passed. When mineral fertilizer was added to the soil total respiration rate had similar values to control treatment. The addition of minerals to the soil did not increase easily mineralizable compounds so catabolic activity was limited (Table 3, 4, 5, 6).

Soil basal respiration ranged from a minimum of 0.28 µg/g/h CO_2-C recorded in mineral treatment after 10 days of incubation to a maximum of 1.21 µg/g/h CO_2-C in slurry treatment measured after 20 days of incubation (Table 6). Overall, the results showed a clear trend of increasing CO_2 production from the beginning to the end of the experiment with the highest values recorded after 20 days of incubation for both mineral and organic fertilized soils. A positive correlation was reported between basal respiration and specific substrate respiration (Figure 1). These results indicate that microbial biomass increased in all treatments during the first 20 days of the experiment. Other studies concluded also that the addition of organic fertilizers enhances microbial biomass which starts to mineralize organic compounds with an increase in CO_2 production (Ge et al., 2013; Heitkamp et al., 2009; Ekelund et al., 2005). However, after 30 days of intense metabolic activity, the reduction of soil N-NO_3 has a negative effect on soil basal respiration (Figure 1).

During the experiment, for all treatments, an increase in specific substrates utilization was reported for most of the used substrates (Table 3, 4, 5, 6). The highest respiration rate was recorded for α-ketoglutaric acid (7.44 µg/g/h CO_2-C) in green manure treatments (Table 3), while the lowest respiration rate was measured for arginine in mineral treatments (0,05 µg/g/h CO_2-C) (Table 5). Creamer et al. (2016) suggest that α-ketoglutaric acid and citric acid are better catabolized in soils with higher pH.

Table 3. Mean catabolic respiration rate (µg/g/h CO_2-C) for specific carboxylic acid substrates in experiment treatments

Treatment	Sampling	Oxalic acid	Ketoglutaric acid	Citric acid	L-malic acid
Co	10 days	1.68 ± 0.11^{a}	4.97 ± 0.49^{a}	4.56 ± 0.05^{a}	1.57 ± 0.05^{gh}
	20 days	1.60 ± 0.03^{a}	3.90 ± 0.41^{ab}	3.52 ± 0.15^{a}	1.79 ± 0.10^{fgh}
	30 days	1.99 ± 0.15^{ab}	6.36 ± 0.30^{ab}	4.54 ± 0.42^{ab}	2.57 ± 0.14^{abcde}
CS	10 days	1.65 ± 0.34^{bc}	4.88 ± 0.27^{ab}	4.40 ± 0.13^{abc}	2.38 ± 0.09^{bcdef}
	20 days	2.13 ± 0.11^{bcd}	4.47 ± 0.06^{bc}	3.81 ± 0.17^{abc}	2.60 ± 0.08^{abcd}
	30 days	1.81 ± 0.09^{cde}	4.45 ± 0.36^{cd}	3.41 ± 0.33^{abcd}	2.16 ± 0.17^{cdefg}
CM	10 days	2.57 ± 0.11^{cdef}	7.03 ± 0.52^{cde}	5.09 ± 0.43^{bcde}	3.10 ± 0.81^{a}
	20 days	2.50 ± 0.09^{cdefg}	6.40 ± 0.58^{de}	4.82 ± 0.49^{bcdef}	2.88 ± 0.15^{abc}
	30 days	1.74 ± 0.06^{cdefg}	3.76 ± 0.30^{def}	3.03 ± 0.08^{cdef}	2.13 ± 0.22^{defgh}
IV	10 days	2.84 ± 0.22^{defg}	7.44 ± 0.49^{def}	5.19 ± 0.29^{def}	2.90 ± 0.12^{ab}
	20 days	2.17 ± 0.12^{efg}	5.94 ± 0.11^{def}	4.02 ± 0.36^{def}	2.57 ± 0.09^{abcd}
	30 days	1.52 ± 0.09^{efg}	3.55 ± 0.43^{def}	3.23 ± 0.05^{ef}	1.86 ± 0.09^{efgh}
M	10 days	1.62 ± 0.11^{fg}	4.33 ± 0.56^{def}	3.46 ± 0.37^{ef}	1.43 ± 0.15^{h}
	20 days	2.04 ± 0.06^{g}	4.20 ± 0.34^{ef}	3.92 ± 0.16^{ef}	2.26 ± 0.15^{bcdefg}
	30 days	1.91 ± 0.02^{g}	4.73 ± 0.36^{f}	3.62 ± 0.55^{f}	2.02 ± 0.22^{defgh}

Data are mean ± S.E. Values followed by the same letter are not significant ($P < 0.05$)

Table 4. Mean catabolic respiration rate (µg/g/h CO_2-C) for specific carbohydrate substrates in the experiment treatments

Treatment	Sampling	Trehalose	Galactose	Arabinose	D-Glucose	D-Fructose
Co	10 days	0.55 ± 0.06^{gh}	0.50 ± 0.01^{a}	0.77 ± 0.05^{a}	0.56 ± 0.06^{a}	0.74 ± 0.10^{a}
	20 days	0.63 ± 0.02^{gh}	0.55 ± 0.04^{ab}	0.77 ± 0.01^{ab}	0.76 ± 0.03^{a}	0.83 ± 0.11^{a}
	30 days	1.41 ± 0.08^{bc}	1.58 ± 0.12^{ab}	1.73 ± 0.11^{abc}	1.47 ± 0.13^{b}	1.79 ± 0.08^{ab}
CS	10 days	1.01 ± 0.17^{de}	0.89 ± 0.06^{ab}	1.22 ± 0.15^{abc}	1.58 ± 0.32^{bc}	1.47 ± 0.20^{bc}
	20 days	1.95 ± 0.14^{a}	1.79 ± 0.06^{ab}	2.03 ± 0.18^{bc}	2.67 ± 0.20^{cd}	2.29 ± 0.17^{cd}
	30 days	1.25 ± 0.11^{cd}	1.34 ± 0.14^{bc}	1.48 ± 0.14^{cd}	1.61 ± 0.07^{cd}	1.63 ± 0.11^{cd}
CM	10 days	0.72 ± 0.03^{fg}	0.77 ± 0.07^{cd}	0.88 ± 0.04^{de}	0.99 ± 0.07^{de}	1.05 ± 0.08^{cde}
	20 days	1.44 ± 0.20^{bc}	1.58 ± 0.32^{cd}	1.81 ± 0.20^{de}	2.05 ± 0.40^{def}	1.69 ± 0.20^{cde}
	30 days	0.96 ± 0.03^{ef}	0.93 ± 0.03^{d}	1.00 ± 0.07^{def}	1.24 ± 0.13^{def}	1.16 ± 0.07^{de}
IV	10 days	1.60 ± 0.14^{b}	1.58 ± 0.14^{d}	1.64 ± 0.02^{ef}	2.61 ± 0.1^{defg}	2.41 ± 0.16^{def}
	20 days	2.09 ± 0.06^{a}	1.57 ± 0.19^{de}	1.99 ± 0.15^{ef}	2.13 ± 0.05^{efg}	2.07 ± 0.14^{efg}
	30 days	1.11 ± 0.05^{de}	1.08 ± 0.08^{def}	1.22 ± 0.10^{efg}	1.23 ± 0.02^{fgh}	1.45 ± 0.06^{fgh}
M	10 days	0.39 ± 0.01^{h}	0.42 ± 0.04^{efg}	0.52 ± 0.05^{fg}	0.48 ± 0.06^{ghi}	0.62 ± 0.06^{ghi}
	20 days	1.16 ± 0.01^{cde}	1.08 ± 0.02^{fg}	1.08 ± 0.32^{fg}	1.25 ± 0.11^{hi}	1.40 ± 0.01^{hi}
	30 days	0.97 ± 0.06^{ef}	0.96 ± 0.06^{g}	1.11 ± 0.04^{g}	1.11 ± 0.05^{i}	1.38 ± 0.04^{i}

Data are mean ± S.E. Values followed by the same letter are not significant ($P < 0.05$)

Table 5. Mean catabolic respiration rate (µg/g/h CO_2-C) for specific aminoacids substrates in the experiment treatments

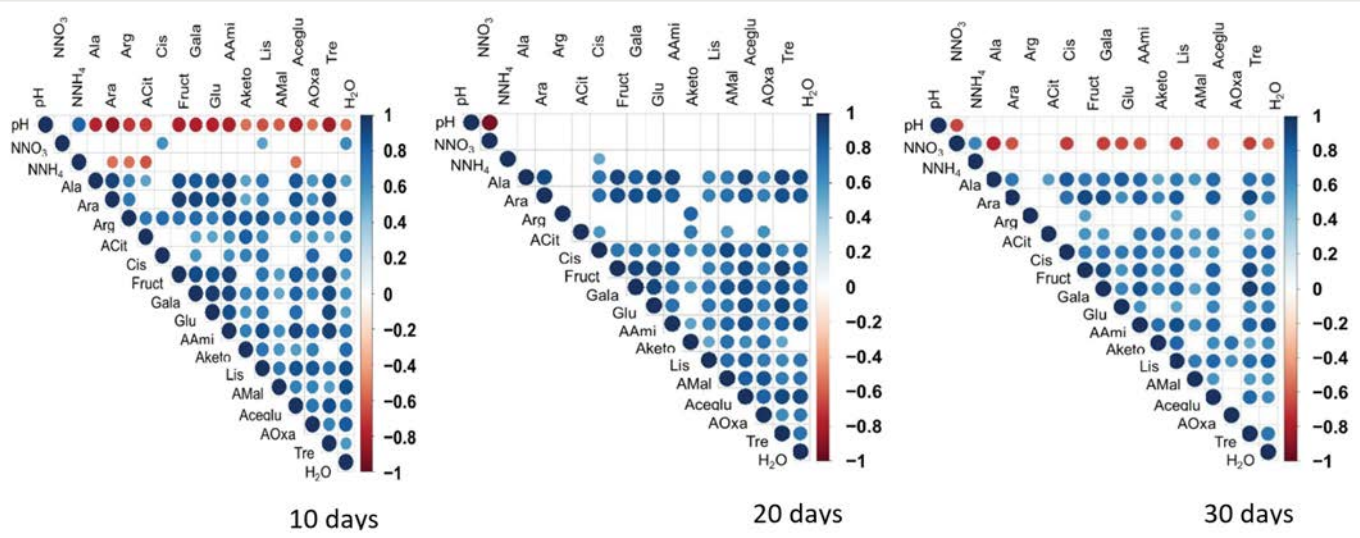
Treatment	Sampling	L-arginine	Aminobutyric acid	L-lysine	L-alanine	L-cysteine
Co	10 days	0.08 ± 0.00^{f}	0.42 ± 0.01^{i}	0.47 ± 0.01^{g}	0.54 ± 0.05^{hi}	0.68 ± 0.03^{g}
	20 days	0.66 ± 0.04^{c}	0.53 ± 0.03^{hi}	0.76 ± 0.16^{ef}	0.55 ± 0.02^{hi}	0.64 ± 0.03^{g}
	30 days	0.46 ± 0.06^{d}	1.26 ± 0.05^{bc}	1.28 ± 0.05^{ab}	1.33 ± 0.08^{bc}	1.44 ± 0.02^{ab}
CS	10 days	0.07 ± 0.00^{f}	0.66 ± 0.09^{gh}	0.62 ± 0.06^{fg}	0.95 ± 0.07^{efg}	0.70 ± 0.02^{g}
	20 days	0.31 ± 0.06^{e}	1.52 ± 0.14^{a}	1.32 ± 0.11^{ab}	1.78 ± 0.12^{a}	1.34 ± 0.08^{abc}
	30 days	0.37 ± 0.02^{de}	0.96 ± 0.04^{def}	1.05 ± 0.03^{cd}	1.20 ± 0.03^{bcd}	1.28 ± 0.12^{bc}
CM	10 days	0.12 ± 0.01^{f}	0.68 ± 0.03^{gh}	0.80 ± 0.02^{ef}	0.73 ± 0.03^{gh}	1.03 ± 0.05^{de}
	20 days	1.40 ± 0.09^{b}	1.40 ± 0.09^{ab}	1.42 ± 0.08^{a}	1.42 ± 0.15^{b}	1.56 ± 0.02^{a}
	30 days	0.31 ± 0.01^{e}	0.77 ± 0.02^{fg}	0.82 ± 0.04^{ef}	1.02 ± 0.10^{def}	1.11 ± 0.07^{cde}
IV	10 days	0.15 ± 0.00^{f}	1.04 ± 0.03^{de}	0.84 ± 0.02^{def}	1.20 ± 0.15^{bcd}	1.02 ± 0.03^{de}
	20 days	1.66 ± 0.10^{a}	1.49 ± 0.10^{a}	1.17 ± 0.12^{bc}	1.70 ± 0.02^{a}	1.29 ± 0.18^{bc}
	30 days	0.41 ± 0.04^{de}	0.85 ± 0.04^{efg}	0.76 ± 0.01^{ef}	0.99 ± 0.06^{def}	0.94 ± 0.03^{ef}
M	10 days	0.05 ± 0.00^{f}	0.36 ± 0.01^{i}	0.48 ± 0.03^{g}	0.50 ± 0.03^{i}	0.75 ± 0.11^{fg}
	20 days	0.30 ± 0.02^{e}	1.07 ± 0.13^{cd}	0.97 ± 0.15^{cde}	1.12 ± 0.09^{cde}	1.17 ± 0.11^{cd}
	30 days	0.38 ± 0.03^{de}	0.82 ± 0.05^{fg}	0.86 ± 0.03^{de}	0.88 ± 0.03^{fg}	1.04 ± 0.06^{de}

Data are mean ± S.E. Values followed by the same letter are not significant ($P < 0.05$)

Table 6. Mean catabolic respiration rate (µg/g/h CO_2-C) for N-acetylglucosamine and water (basal respiration) in the experiment treatments

Treatment	Sampling	N-acetylglucosamine	Water
Co	10 days	0.47 ± 0.02^{h}	0.29 ± 0.01^{h}
	20 days	0.57 ± 0.04^{h}	0.36 ± 0.00^{gh}
	30 days	1.37 ± 0.01^{bc}	1.00 ± 0.10^{b}
CS	10 days	1.12 ± 0.22^{def}	0.36 ± 0.04^{gh}
	20 days	1.61 ± 0.09^{a}	1.21 ± 0.16^{a}
	30 days	1.24 ± 0.04^{cde}	0.80 ± 0.08^{cd}
CM	10 days	1.02 ± 0.04^{efg}	0.57 ± 0.06^{ef}
	20 days	1.54 ± 0.06^{ab}	1.10 ± 0.06^{ab}
	30 days	0.85 ± 0.07^{g}	0.63 ± 0.02^{def}
IV	10 days	1.31 ± 0.08^{bcd}	0.52 ± 0.01^{fg}
	20 days	1.53 ± 0.10^{ab}	0.94 ± 0.09^{bc}
	30 days	1.04 ± 0.03^{efg}	0.55 ± 0.01^{ef}
M	10 days	0.37 ± 0.02^{h}	0.28 ± 0.01^{h}
	20 days	1.07 ± 0.00^{efg}	0.74 ± 0.06^{de}
	30 days	0.96 ± 0.11^{fg}	0.69 ± 0.06^{def}

Data are mean ± S.E. Values followed by the same letter are not significant ($P < 0.05$)



Note: Blue and red colour dots represent positive and negative correlation

Figure 1. Soil correlogram showing the relationship between soil parameter (pH, N-NO_3, N-NH_4) and substrate utilization after 10, 20 and 30 days of incubation

Contrary to this result, even if the measured pH was above 7, a negative effect of pH was revealed by correlogram after 10 days of incubation. We only speculate that this result was obtained as a consequence of high pH variation in treatment experiments after the addition of fertilizers to the soil (Figure 1). This negative effect was not present after the first sampling measurements. Kemmit et al. (2006) indicated nutrient availability as a driver for increasing metabolic activity and Rutgers et al. (2016) reported a strong correlation of carboxylic acids with arable soils. Romaniuk et al. (2011) suggest that r-strategy microorganisms can metabolise α-ketoglutaric acid and other carboxylic acids indicating that the presence of high C: N organic matter in soil favours the development of this group.

The catabolic response pattern for all treatments was similar, with the greatest metabolic activity recorded for carboxylic acids, followed by carbohydrates, amino sugars and amino acids. Correlation indices were mainly positive for each sampling date showing that variables have a positive effect on microbial community metabolism. Similar results were presented by Esperschütz et al. (2007) who reported also a more diverse and metabolically active community in the organic fertilizer system. These results are similar to the results obtained in our experiment where the pH of the soil was slightly basic and the availability of nitrogen was high.

CONCLUSIONS

Our results showed that microbial metabolic activity was affected by the type of fertilizers and date of analyses. The use of organic amendments has an overall positive influence on the catabolic rates, with the mean C-CO_2 released from soil having the highest value for green manure, followed by cattle manure, slurry manure, control and mineral treatments.

The microbial physiological responses were more sensitive to cattle manure application during the first part of the experiment and to green manure (mustard) presence after 10 days of incubation. As a consequence, soil nitrogen mineralization follows the same trends with high quantities released during the first 10 days in the case of slurry and cattle manure treatments. When green manure was used as organic amendments nitrogen mineralization was more intense between 10 and 20 days of incubation. The presence of mineral fertilizers in soil caused a decrease in microbial metabolic activity with limited effect on mineralization. These results suggest that fertilizer applications should consider the mineralization potential of different organic amendments and microbial metabolic activity to avoid soil nutrient loss and to improve soil fertility.

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