

## Organic materials: sources of nitrogen in the organic production of lettuce

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Received: 08.05.2009

**Abstract:** This paper presents the results of 2 experiments: an incubation experiment and a subsequent field experiment. An incubation experiment was set up in order to determine the mineralization potential of different organic materials (OMs) (well-rotted farmyard manure [FTM], guano [G], soybean seed [S], and forage pea seed [P]), the kinetics of mineral nitrogen (N) release, and the correlation between OM content and the quantity of mineralized N. The results of the incubation experiment were checked under field conditions in which different OMs were used as N sources for lettuce (*Lactuca sativa* L.). The highest mineralization rate constant  $k$  ( $0.127 \text{ day}^{-1}$ ) and net mineralization rate (NM) (76.37% of total applied N) were obtained with G, and the lowest  $k$  ( $0.098 \text{ day}^{-1}$ ) and NM (30.12% of total applied N) were obtained with FYM. In the field experiment in 2007 and 2008 we applied different OM sources that contained the same amount of potentially mineralizable N (40 and 35 kg N ha<sup>-1</sup>, respectively), calculated on the basis of  $k$  and NM. The use of OMs increased the concentration of mineral N in the soil and increased the yield of fresh lettuce. Nitrate content in the fresh lettuce was within acceptable limits both years (<2500 mg kg<sup>-1</sup>). Total N in the above-ground parts of lettuce ranged from 44.38 kg N ha<sup>-1</sup> (Ø, treatment without fertilization) to 67.45 kg N ha<sup>-1</sup> (S) (2-year average). The results show that when determining the quantity of OM to apply it is necessary to take into account the quantity of potentially mineralizable N in order for the plants to use N as efficiently as possible, to regulate the nitrate content in fresh lettuce, and to control the quantity of residual mineral N in the soil at the end of the vegetation period.

**Key words:** Lettuce, nitrogen, organic farming, potentially mineralizable N

**Abbreviations:** OM: organic material; G: guano; S: soybean seed; P: forage pea seed; NM: net mineralization;  $k$ : mineralization rate constant;  $A_N$ : potentially mineralizable N

### Introduction

Organic farming excludes the use of N mineral fertilizers (FAO Codex Alimentarius 1999); therefore, supplying crops with N requires the use of organic sources of N—organic matter in the soil and the application of different organic materials (OMs), such

as organic fertilizers, legumes, and crop residue. As mineralization is biological decomposition, the availability of N for crop plants is controlled by the chemical composition of OM, and the physical, chemical, and biological characteristics of the soil (Vigil and Kissel 1995; Pansu and Thuries 2003).

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By plowing under crop residue (Trinsoutrot et al. 2000; Chaves et al. 2004), applying organic waste and manure with relatively high N content (>1.5%) and a narrow C/N ratio (<20) (Cordovil et al. 2005; Burgos et al. 2006), and via their mineralization in the soil and significant N content of its mineral, the available form of N can be released, satisfying crop requirements for N (Amlinger et al. 2003; Bavec et al. 2006). On the other hand, materials with a wider C/N ratio cause immobilization of N (Chaves et al. 2005), which in turn reduces the yield of short vegetation crops. As such, it is necessary, when using organic sources of N, to estimate the mineralization potential of OMs so that by selecting a fertilizer with an appropriate content and by applying it at different times, the needs of the crops can be synchronized with the release of nutrients (Pang and Letey 2000; Berry et al. 2002).

Excessive and inappropriate use of OMs can cause intensive mineralization and drastically increase mineral N in the soil and plants (Burns 1996), which triggers various disturbances in the biological equilibrium of the ecosystem, and excessive and harmful accumulation of nitrates in the plants (Andersen and Nielsen 1992).

Lettuce (*Lactuca sativa* L.) is one of the most popular vegetable species, characterized by the accumulation of high amounts of nitrates in the fresh mass. In organic production, which is traditionally performed on small family farms, the production of lettuce is of great economic and social importance (Čabilovski et al. 2008; Porto et al. 2008). As organic farming in southeast Europe is increasing in

importance (Willer and Yussefi 2006; Al-Bitar 2008) and there are not enough data on OMs that could be sources of N (Cuvaradic et al. 2006), the goals of the present study were as follows:

To determine the mineralization potential of different OMs, the kinetics of mineral N release, and the correlation between OM contents and the quantity of mineralized N by means of an incubation experiment;

To estimate the quantities of different OMs needed to satisfy N needs in field-grown lettuce (*Lactuca sativa* L.), based on the potentially mineralizable N content;

To examine under field conditions the effects of the applied OMs on lettuce yield, on nitrate content in fresh mass, and residual N content in the soil.

## Materials and methods

### Amendment and plant material characterization

The chemical composition of the applied OMs is given in Table 1. Dry matter content was determined gravimetrically (70 °C for 24h). Total C and N content was determined using a CHNS analyzer (Elementar Vario EL, GmbH, Hanau, Germany). Mineral N (NH<sub>4</sub>-N and NO<sub>3</sub>-N) content in the OMs was determined after extraction in water, using the steam distillation method (Keeney and Nelson 1982). Nitrate content in lettuce, following distilled water extraction and application of phenol disulfonic acid and ammonium hydroxide, was determined by the spectrophotometric method ( $\lambda$ , 420) (Johnson and Ulrich 1950).

Table 1. Chemical composition of the OMs used in the experiment.

Organic material	DM <sup>1</sup> (%)	Total C (%)	Total N (%)	WSN <sup>2</sup> (percent of total N)	C/N ratio
FYM	88.86	22.80	2.00	2.45	11.40
G	93.28	44.40	15.32	0.96	2.89
P	96.20	41.55	4.08	0.17	10.17
S	96.80	49.93	6.65	0.23	7.50

<sup>1</sup>DM: dry matter; <sup>2</sup>WSN: water-soluble N

### Soil data and soil characterization

The soil used in both field incubation experiments was non-calcareous chernozem. A soil sample was taken from the topsoil layer (0-30 cm) of a farm in Kisač, Serbia (lat 45°35'N, long 19°72'E; 85 m altitude a.s.l.), which was in the process of being certified for organic production. The pH value of the soil was determined in a suspension of soil and H<sub>2</sub>O (1:2.5) using a Metrel MA 3657 pH meter. CaCO<sub>3</sub> content was determined volumetrically using a Scheibler calcimeter (Soil Survey Staff 1993). Total N content was determined using a CHNS analyzer (Elementar Vario EL, GmbH, Hanau, Germany). Organic C content was determined by oxidizing the OMs with potassium dichromate (Simakov and Tsyplemkov 1969). Plant available P and K were extracted with an AL solution (0.1 M ammonium lactate and 0.4 M acetic acid, pH 3.75) at a soil to solution ratio of 1:20 (w/v) (Enger et al. 1960). The concentration of P was measured by spectrophotometry, while the concentration of K was measured by flame photometry. Mineral N content under field conditions during the vegetation of lettuce was determined by the Wehrmann and Scharpf method (1979). Basic chemical characteristics of the soil were as follows: pH, 7.52 (in H<sub>2</sub>O); 0.17% CaCO<sub>3</sub>; 3.31% humus; 0.227% total N; 11.02 C/N ratio; 11.21 mg 100 g<sup>-1</sup> AL-P<sub>2</sub>O<sub>5</sub>; 26.80 mg 100 g<sup>-1</sup> AL-K<sub>2</sub>O.

### Incubation procedure

Four different OMs were analyzed in the incubation experiment: well-rotted farmyard manure (FYM), guano (G), soybean seed, *Glycine hispida* (S), and forage pea seed, *Pisum sativum* (P). PVC containers (100 cm<sup>3</sup>) were filled with soil (with 70% of holding water capacity, which corresponds to the mass of 50 g of absolutely dry soil) mixed with ground OM, which was added in the amount that incorporated 5 mg of total N. Then the containers were covered with semi-permeable wax parafilm in order to reduce water loss during incubation. Moisture in the soil was maintained at the same level by weighing the mass of the container every 7 days and adding distilled water when the mass dropped by more than 0.05 g. Incubation in the dark lasted for 28 days and at the constant temperature of 28 °C. A sample of soil without added OM was incubated as a control so that we could assess the soil's

mineralization potential and calculate the portion of mineralized N in total N contained in OM. Mineral N content in the containers was measured every 7 days by taking 4 repetitions of every treatment. Mineral N in the soil was extracted by 2 M KCl (1:4 soil to solution ratio, weight basis) and determined by steam distillation (Keeney and Nelson 1982).

Net mineralization (NM) of the OMs was calculated as the difference between mineral N content in the containers with OM and without OM (soil only) using the following equation:

$$NM(\%) = \frac{T-K-P}{V} \times 100\% \quad (1)$$

where NM is net mineralization of OM, T is mineral N content in the container with OM (mg of NH<sub>4</sub>-N + NO<sub>3</sub>-N), K is mineral N content in the container without OM (mg of NH<sub>4</sub>-N + NO<sub>3</sub>-N), P is initial mineral N content, and V is total N content in OM (5 mg N container<sup>-1</sup>).

The net mineralization patterns of the OMs were fitted to the single first-order kinetics model (Stanford and Smith 1972).

$$A_{N(t)} = A_N(1 - \exp^{-kt}) \quad (2)$$

where  $A_{N(t)}$  is the amount of mineral N released at time  $t$  (percent of total N applied),  $A_N$  is the amount of potentially mineralizable N (percent of total N applied), and  $k$  is the N mineralization rate constant (day<sup>-1</sup>).

The zero order kinetics model  $N_{(t)} = k t$  ( $N_{(t)}$  is the amount of N mineralized after time  $t$ ) was used to describe N mineralization from the blank soil (De Neve et al. 1996).

### Field experiment

The effects of different OMs tested in the incubation experiment were studied in the vegetation field experiment on leafy lettuce (*Lactuca sativa* L.), var. Brezolist, as the principal crop. The experiment was conducted during 2007 and 2008 on the plot from which the soil samples were taken for the incubation experiment. Mean weekly air temperature, total precipitation, and soil moisture of the root system (0-30 cm) for each week are given in Table 2.

Table 2. Mean weekly air temperature, total precipitation, and soil moisture in the area of the roots during the vegetation of lettuce.

Parameters	Year	Vegetation period (weeks)								
		1	2	3	4	5	6	7	8	9
Air temperature (°C)	2007	9.9	6.9	10.6	11.7	11.4	13.5	17.2	20.5	-
	2008	7.2	6.1	10.1	13.1	12.3	14.6	14.0	17.7	19.6
Precipitation (mm)	2007	0	45.4	0.1	0	0	52.7	0	0.7	-
	2008	11.2	5.3	0.2	4.10	6.4	2.1	25.9	0.4	5
Soil moisture (%) <sup>1</sup>	2007	88.5	92.5	90.5	89.9	82.5	90.1	80.1	75.6	-
	2008	87.0	89.5	90.6	89.4	84.7	83.4	77.3	76.1	76.9

<sup>1</sup>Percent of field water capacity

In order to avoid the prolonged effect of applied OM and intercropping, the experiment was set up on 2 different parts of the same plot during the 2 years. It was set up using a block system with random distribution of treatments in 4 replications. The treatments were as follows: control (Ø1), farmyard manure (FYM), guano (G), forage pea seed (P), and soybean seed (S). In addition to these treatments, we observed the content of mineral forms of N during the vegetation of lettuce (Ø2) in the plots without vegetation.

Total quantity of applied OMs differed in the 2 years, depending on the amount of mineral forms of N in the soil at the time of planting. OMs were applied during the preparation of the soil, immediately before planting the lettuce in the open field. Soybean seed and forage pea seed were previously ground and sifted through a 2-mm sieve. Soil moisture during vegetation was maintained at an optimal level using a

Tifon irrigation system. The main plot was 5.4 m<sup>2</sup> (1.8 × 3 m). Table 3 provides basic data on the timing of technological operations during the production of the field-grown lettuce.

Soil samples used to determine mineral N content during lettuce vegetation were 2 individual samples taken from every treatment replication. During harvesting the mass of 30 plants from each replication was measured to establish total yield, whereas the sample for determining nitrate content in fresh mass was 4 lettuce plants.

#### Statistical analysis

Results were subjected to analysis of variance (ANOVA), according to their experimental design, and treatment mean results were compared using Tukey's test ( $P < 0.05$ ). The analyses were performed using STATISTICA v.7.0.

Table 3. Dates of procedures during lettuce production.

Year	Procedure					
	Lettuce sowing <sup>1</sup>	OM application	Lettuce planting <sup>2</sup>	Agril foil removing	Lettuce harvest <sup>3</sup>	Vegetation (days)
2007	7 February	17 March	18 March	18 April	12 May	56
2008	12 February	15 March	17 March	23 April	18 May	63

<sup>1</sup> Lettuce seed was sown in plastic containers with a nutritious substrate. Young lettuce plants were grown in the greenhouse until they formed 4-6 leaves.

<sup>2</sup> Planting density (11 plants m<sup>-2</sup>).

<sup>3</sup> Lettuce was harvested when it was commercially ripened

## Results

### Net nitrogen mineralization

Net OM mineralization values are given in Table 4. The highest NM values were obtained with treatment G (76.37%), while significantly lower values were obtained with S (41.23%) and P (36.1%). The lowest NM value was obtained with the FYM treatment (30.12%). The difference in NM between treatments P and S at the end of incubation was not statistically significant (Table 4).

Using the zero order kinetics model ( $N_{(t)} = k t$ )  $k$  and  $N_t$  values were calculated for blank soil. The N mineralization rate constant of the blank soil was 0.09 mg N 100 g<sup>-1</sup> soil day<sup>-1</sup> and the total amount of

mineralized N ( $N_t$ ) from the soil's organic matter was 1.88 mg N 100 g soil<sup>-1</sup>, which corresponds to 66 kg N ha<sup>-1</sup> for topsoil (0-30 cm).

Total N content ( $N_{tot}$ ) and the C/N ratio of the OMs were compared with the parameters of the kinetic model,  $A_N$  and  $k$ . Statistically significant correlations were observed between mineralization parameters ( $A_N$  and  $k$ ) and between chemical composition parameters ( $N_{tot}$  and C/N ratio) (Table 5).

### Calculation of N needed for the nutrition of organically produced lettuce

Poovatomdom (1988), Sierra (1997), and Katterer et al. (1998) reported that at temperatures ranging from

Table 4. Recorded NM of analyzed OMs at the end of the incubation period and parameters of the kinetic model  $A_N$  and  $k$ .

Organic Materials	$k^1$ (day <sup>-1</sup> )	$A_N^2$ (%)	NM <sup>3</sup> (%)	$r^4$
FYM	0.098	30.40	30.12 c	0.78
G	0.127	72.69	76.37 a	0.87
P	0.114	36.60	36.10 b	0.85
S	0.119	40.28	41.23 b	0.82

<sup>1</sup>  $k$ : Mineralization rate constant;

<sup>2</sup>  $A_N$ : amount of potentially mineralizable N (percent of total N applied) according to the kinetic model;

<sup>3</sup> NM: recorded net mineralization of OM (percent of total N applied);

<sup>4</sup>  $r$ : correlation coefficient between NM and  $A_N$ . Values followed by different letters are significant at  $P < 0.01$

Table 5. Correlation coefficients and equations of regression between chemical composition of OM mineralization parameters.

Mineralization parameters	Chemical composition	$r$	Regression equations
$A_N^1$	C/N ratio	-0.97*	-5.8026 C/N + 90.896
	$N_{tot}^3$	0.99*	3.5117 $N_{tot}^3$ + 21.285
$k^2$	C/N ratio	-0.87	-0.0005 C/N <sup>2</sup> + 0.0037 C/N + 0.12
	$N_{tot}^3$	0.98*	-0.0003 $N_{tot}^2$ + 0.0071 $N_{tot}$ + 0.08

<sup>1</sup>  $A_N$ : Amount of potentially mineralizable N (percent of total N applied), values of NM according to the kinetic model;

<sup>2</sup>  $k$ : mineralization rate constant;

<sup>3</sup>  $N_{tot}$ : total amount of N in OM (%);

\*: values with superscripts are statistically significant ( $P < 0.05$ )

5 to 35 °C if the temperature rises 10 °C the level of mineralization of OM in soil doubles. Considering the fact that the vegetation period of lettuce in field conditions was 8 weeks, and that the average air temperature during vegetation was 13 °C (20 March-20 May), we estimated that the mineralized of N from the OMs and organic matter in the soil would be equivalent to values obtained in the incubation experiment, which lasted only 4 weeks at a temperature almost 2-fold higher (28 °C).

Based on the premise that the total N requirement of lettuce was no more than 120 kg N ha<sup>-1</sup> (Doerge et al. 1991; Sorensen et al. 1994; Bavec 2003) and using Eq. (3), we calculated the total quantity of N from the quantity of potentially mineralizable N required to satisfy lettuce that was incorporated into the soil (40 and 35 kg N ha<sup>-1</sup>) (Table 6).

$$N_f = (N_{tg} - N_i - N_{pot} - N_w + N_l)/b \quad (3)$$

where  $N_f$  is the amount of N applied through different OMs (kg N ha<sup>-1</sup>),  $N_{tg}$  is total need for N (120 kg N ha<sup>-1</sup>),  $N_i$  is mineral N content in the soil at the time of planting (32 kg N ha<sup>-1</sup> in 2007; 35 kg N ha<sup>-1</sup> in 2008),  $N_{pot}$  is the amount of mineral N that will be released by the mineralization of organic matter in the soil during lettuce vegetation (66 kg N ha<sup>-1</sup>),  $N_w$  is the amount of N introduced by irrigation (7 kg N ha<sup>-1</sup>),  $N_l$  is N losses (volatilization, denitrification, and leaching) (25 kg N ha<sup>-1</sup>) estimated according to data in the literature, and  $b$  is the coefficient of the availability of total N applied via different OMs (potentially mineralizable N) ( $b = A_N/100$ ).

### Mineral forms of N in the soil during the vegetation period of lettuce

As a result of the mineralization of organic matter in the soil and of the applied OMs, the content of mineral forms of N in the soil increased significantly more with fertilized treatments, as compared to the control for most of the vegetation period both years of the study.

At the time of harvesting mineral N content in the soil in plots without vegetation in the first and second years of the study was 53 and 78 kg N ha<sup>-1</sup>, respectively (Figure 1). If we subtract the initial amounts of mineral N present in the soil at the moment of planting the lettuce in the open field we get net mineralization of 30 and 43 kg N ha<sup>-1</sup> for the first and second year, respectively.

### The effect of the application of OM on the yield and nitrate content in lettuce

Applied OMs had a significant effect on the total yield of lettuce. Both years the yield of fresh mass of lettuce after the OM treatments was significantly higher than that obtained with the control treatment, while the difference in yield between the 2 years was not statistically significant (Table 7).

Nitrate content in the fresh mass of lettuce obtained with all treatments both years was below the maximum allowed content (2500 mg kg<sup>-1</sup>), which is regulated by the EU (Commission Regulation (EC) No 466/2001, Annex 1, Section 1). In both years the lowest nitrate content in the fresh mass of lettuce was obtained with the control treatment (Ø) and the highest was obtained with the S treatment. Nitrate

Table 6. Applied amounts of OM and total N (in 2007 and 2008).

Organic Materials	OM applied (kg ha <sup>-1</sup> )		Total N applied <sup>1</sup> (kg N ha <sup>-1</sup> )	
	2007	2008	2007	2008
FYM	7500	6600	150	132
G	391	338	60	52
P	2941	2576	121	106
S	1624	1429	108	95

<sup>1</sup> Through the applied amounts of total N, 40 kg N ha<sup>-1</sup> (in 2007) and 35 kg N ha<sup>-1</sup> (in 2008) of potentially mineralizable N was incorporated into the soil

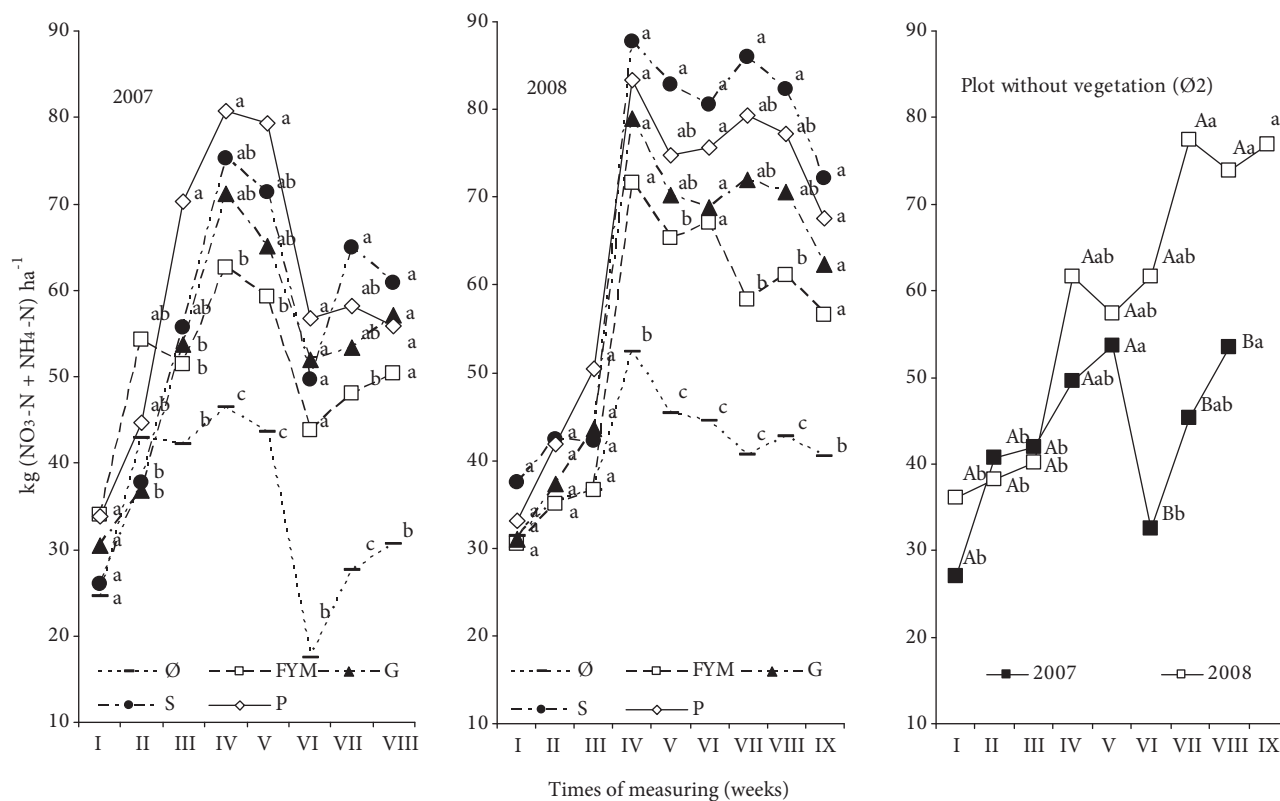


Figure 1. Content of mineral N ( $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$ ) in the soil (0-30 cm) during the lettuce vegetation (in 2007 and 2008).

Table 7. Lettuce yield, nitrate content in fresh mass of lettuce, amount of N taken up, and mineral N content in the soil ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) at the time of harvesting (2007 and 2008).

Treatment <sup>2</sup>	Yield (kg FM <sup>1</sup> ha <sup>-1</sup> )		Nitrate (mg $\text{NO}_3$ , kg <sup>-1</sup> )		N-uptake (kg N ha <sup>-1</sup> )		Min N-soil (kg N ha <sup>-1</sup> )	
	2007	2008	2007	2008	2007	2008	2007	2008
Ø	38711b	39975b	1487b	1134c	45.8b	50.6b	30,7b	40,5c
FYM	45843a	46927a	1642b	1579bc	63.8a	67.3a	50.4a	56.6b
G	44148a	47773a	2103a	1970ab	66.6a	73.5a	57.1a	64.9a
P	43666a	47229a	2207a	2104a	66.6a	67.2a	55.8a	72.0a
S	45416a	46653a	2406a	2258a	73.4a	71.9a	61.0a	68.0a
Average	43557A	45711A	1969A	1809A	63.2A	66.1A	51.0A	60.4A

<sup>1</sup> FM: Fresh mass;

<sup>2</sup> Ø: control; FYM: farmyard manure; G: guano; P: forage pea seed; S: soybean seed. Values followed by different upper- and lowercase letters are significant at  $P < 0.05$ . Uppercase letters denote differences between the years and lowercase letters denote differences between times of measurement in each year. Year  $\times$  time interactions were not statistically significant

content obtained with the G, P, and S treatment was significantly higher than that obtained with FYM and Ø, whereas the difference between FYM and Ø was not significant both years of the study.

Mineral N content in the soil and total N in the yield at the moment of harvesting were significantly higher with fertilized treatments than with the control treatment.

## Discussion

OMs analyzed in the incubation experiment exhibited different mineralization dynamics and were dependent on the amount of total N and the C/N ratio, which is in agreement with previous results (Fox et al. 1990; Palm and Sanchez 1991; Quemada and Cabrera 1995). The values of the constant of the mineralization rate of OM,  $k$ , which indicates the rate of mineralization, ranged from  $0.098 \text{ day}^{-1}$  (FYM) to  $0.127 \text{ day}^{-1}$  (G). The values of net OM mineralization calculated on the basis of the kinetic model ( $A_N$ ), which represent the share of potentially mineralizing N in total N, were proportionate to the mineralization rate constant  $k$ , so that the highest  $A_N$  value was obtained with the G treatment (72.69%) and the lowest was obtained with FYM (30.40%).

We observed a significant positive correlation between  $k$  and total N content, while the correlation between  $k$  and the C/N ratio in OMs was negative (Table 5). Similar correlations between chemical composition (C/N ratio and  $N_{\text{tot}}$ ) and mineralization parameters ( $k$  and  $A_N$ ) were reported by Chaves et al. (2004), Cordovil et al. (2005), and Agehara and Warncke (2005).

The determined value of the constant  $k$  ( $0.090 \text{ day}^{-1}$ ) of the rate of mineralization of organic matter present in the soil was higher than the values determined by other researchers that analyzed soils with lower total N content. For example, Cordovil et al. (2005) reported  $k$   $0.032 \text{ day}^{-1}$  for a sandy soil. De Neve et al. (1996), depending on the temperature of incubation, reported the following values for  $k$ :  $0.006 \text{ day}^{-1}$  ( $5.5 \text{ }^\circ\text{C}$ ),  $0.008 \text{ day}^{-1}$  ( $10 \text{ }^\circ\text{C}$ ),  $0.017 \text{ day}^{-1}$  ( $16 \text{ }^\circ\text{C}$ ), and  $0.027 \text{ day}^{-1}$  ( $25 \text{ }^\circ\text{C}$ ). The relatively high  $k$  values for organic matter in the soil in the present study can be explained by the fact that during the incubation we used topsoil with high humus content, as well as the fact that the plot from which the sample was taken was on a farm certified for organic production, where organic fertilizers had been used for years. This might have affected microbiological activity, causing more intensive mineralization of organic matter in the analyzed soil. According to Tu et al. (2006), the soil microbial respiration rate and net N mineralization were, on average, 83% higher for soils in transition from conventional to organic production than for the conventionally used soils.

The content of mineral forms of N in the soil during vegetation exhibited equilibrium between the mineralization of applied OMs and organic matter in the soil on the one hand, and immobilization of mineral N by lettuce plants and the soil's microorganisms on the other. The production of dry matter in lettuce and N uptake followed a square function; N uptake starts to increase considerably from mid-vegetation to harvest time (De Pinheiro Henriques and Marcelis 2000; Broadley et al. 2003). In the second half of the vegetation period, although mineralization conditions were more favorable, mineral N content in the soil actually decreased, primarily as the result of N uptake by the lettuce, which surpassed mineralization (results not shown).

In 2007 the decrease in mineral N content during the second half of vegetation may have been due not only to the process of immobilization, but also to high precipitation during this period, when in a relatively short time there was over 70 mm of rainfall, which could have caused some of the mineral N in the topsoil, i.e. the area where the root system of the lettuce is, to be transferred to deeper layers of the soil. This seems to be supported by the dynamics of mineral N during vegetation of lettuce in the plots without vegetation (Figure 1).

The application of OMs and their mineralization in the soil caused the release of additional quantities of N, which, in addition to having a beneficial effect on the yield, also resulted in a significant increase in nitrates in fresh mass of lettuce (Table 7). Lettuce is a vegetable with a relatively high nitrate content in its fresh mass, even above  $10,000 \text{ mg kg}^{-1}$  (Corré and Breimer 1979; Terbe et al. 1986). A direct effect of the mineral N concentration in the nutritious substrate on the nitrate content in plants has been reported by a number of researchers (Ilin et al. 2000; Broadley et al. 2003; Safaa and Abd El Fattah, 2007). However, the fact that the nitrate content in plants is not affected only by the content of mineral N in the soil is supported by the fact that in the present study the level of nitrates in the fresh mass was lower in 2008 than in 2007, even though the concentrations of mineral N in the soil in all treatments in 2008 were higher than those in 2007. In 2008 lettuce was harvested a week earlier than in 2007; therefore, during the last week of vegetation the plants were



exposed to considerably different conditions, primarily to different temperatures (Figure 1), which could also affect nitrate content in the fresh mass.

In both years of the present study total N taken up by lettuce plants was significantly higher with fertilized treatments than with the control treatment, primarily as a result of significantly higher yields that were obtained with the fertilized treatments (Table 7). The content of mineral N left in the soil was also significantly higher with the fertilized treatments than with the control treatment.

In conclusion, the analyzed OM<sub>s</sub> differed in chemical composition, which affected the rate of their mineralization and NM values at the end of incubation. Automatically, they are not as valuable as N fertilizers. The results show that guano, applied in the amount that incorporated 2.5 times less total N than manure, released the same amount of mineral N as manure. This indicates that, by determining the content of potentially mineralizable N and the OM mineralization rate constant  $k$ , we can significantly improve the efficiency with which

plants use N. The effect that the chemical composition of OM<sub>s</sub> have on their mineralization needs to be taken into account when calculating the doses and time of application, in order to achieve the highest possible efficiency of N utilization by plants, and to regulate the nitrate content in the fresh mass of lettuce and control the amount of residual mineral N in the soil at the end of vegetation. Furthermore, when determining the required amount of N fertilizer it is necessary to assess not only potentially mineralizable N content in OM<sub>s</sub>, but also the sensitivity to temperature change during mineralization of every OM, so that the results obtained by the incubation method could be more accurately implemented in the creation of a universal fertilization model in organic production.

### Acknowledgments

This work was part of a research project funded by the Republic of Serbia, Ministry of Science (projects numbers TR 6906B and 20088). Financial assistance from the Ministry is gratefully acknowledged.

### References

- Agehara S, Warncke DD (2005) Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. *Soil Sci Soc Am J* 69: 1844-1855.
- Al-Bitar L (2008) Organic farming in the Mediterranean: towards further development. Mediterranean Agronomic Institute of Bari. CIHEAM Analytical notes (<http://portail2.reseau-concept.net/Upload/ciheam/fichiers/ANP30.pdf>).
- Amlinger F, Götz B, Dreher P, Geszti J, Weissteiner C (2003) Nitrogen in biowaste and yard waste compost: Dynamics of mobilisation and availability: a review. *European Journal of Soil Biology* 39: 107-116.
- Andersen L, Nielsen NE (1992) A new cultivation method for the production of vegetables with low content of nitrate. *Scientia Horticulturae* 49: 167-171.
- Bavec M, Koren M, Fekonja M, Grobelnik-Mlakar S, Bavec F (2006) Test plant-derived organic fertilizers in different vegetables. In: *Proceeding of the IX ESA Congress* (Eds. M Fotyma, B Kamińska), Vol. 1, Warsaw, Poland, pp. 361-362.
- Bavec M (2003) Tehnike pridelovanja zelenjadnic. Fakulteta za kmetijstvo, Maribor. (In Slovenian)
- Berry PM, Sylvester-Bradley R, Philipps L, Hatch DJ, Cuttle SP, Rayans FW, Gosling P (2002) Is the productivity of organic farms restricted by the supply of available nitrogen? *Soil Use and Management* 18: 248-255.
- Broadley MR, Seginer I, Burns A, Escobar-Gutierrez AJ, Burns IG, White PJ (2003) The nitrogen and nitrate economy of butterhead lettuce (*Lactuca sativa* var. *capitata* L.). *J Exp Bot* 54: 2081-2090.
- Burgos P, Madejón E, Cabrera F (2006) Nitrogen mineralization and nitrate leaching of a sandy soil amended with different organic wastes. *Waste Management & Research* 24: 175-182.
- Burns IG (1996) Nitrogen supply, growth and development. *Acta Horticulturae* 428: 21-30.
- Čabrilovski R, Manojlović M, Bogdanović D, Bavec M (2008) Economic aspects of the application of different organic materials as N-sources in organic production of lettuce. In: *Proceedings of the 16<sup>th</sup> IFOAM Organic World Congress* (Eds. D Neuhoff, N Halberg, T Alföldi, W Lockeretz, A Thommen, IA Rasmussen, J Hermansen, M Vaarst, L Lueck, F Caporali, HH Jensen, P Migliorini, H Willer) Vol. 1, Modena, Italy, pp. 150-154.
- Chaves B, De Neve S, Boeckx P, Van Cleemput O, Hofman G (2005) Screening organic biological wastes for their potential to manipulate the N release from N-rich vegetable crop residues in soil. *Agriculture, Ecosystems and Environment* 111: 81-92.
- Chaves B, De Neve S, Hofman G, Boeckx P, Van Cleemput O (2004) Nitrogen mineralization of vegetable root residues and green manures as related to their (bio)chemical composition. *Europ J Agronomy* 21: 161-170.

- Commission Regulation (EC) (2005) Commission Regulation No 1822/2005 amending Regulation (EC) No 466/2001 as regards nitrate in certain vegetables. Official Journal of the European Union L 293/11.
- Cordovil CM, Coutinho J, Gross M, Cabral F (2005) Potentially mineralizable nitrogen from organic materials applied to a sandy soil: Fitting the one-pool exponential model. *Soil Use and Management* 21: 65-72.
- Corré NM, Breimer T (1979) Nitrate and nitrite in vegetables. Center for Agricultural Publishing and Documentation, Wageningen, Netherlands.
- Cuvardic M, Seremesic S, Novakovic N (2006) Soil fertility in organic farming in the first years after transition. Proceedings of the Joint Organic Congress, Odense, Denmark (<http://orgprints.org/7362/>).
- De Neve S, Pannier J, Hofman G (1996) Temperature effects on C- and N-mineralization from vegetable crop residues. *Plant Soil* 181: 25-30.
- De Pinheiro Henriques AR, Marcelis LFM (2000) Regulation of growth at steady-state nitrogen nutrition in lettuce (*Lactuca sativa* L.): Interactive effects of nitrogen and irradiance. *Annals of Botany* 86: 1073-1080.
- Doerge TA, Roth RL, Gardner BR (1991) Nitrogen fertilizer management in Arizona. Publication number 191025, College of Agriculture, The University of Arizona, Tucson, AZ.
- Enger H, Riehm H, Domingo WR (1960) Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. *Kunl Lantbr Högsk Ann* 26: 199-215.
- FAO (1999) Codex Alimentarius Commission procedural manual (11th ed.). Rome, Italy.
- Fox RH, Myers RJK, Vallis I (1990) The nitrogen mineralization rate of legume residues in soil as influenced by their polyphenol, lignin, and nitrogen contents. *Plant Soil* 129: 251-259.
- Ilin Ž, Đurovka M, Marković V, Lazić B, Bošnjak Đ (2000) Effect of mineral nitrogen concentration in soil and irrigation on NO<sub>3</sub> content in potato tubers. *Acta Hort* 533: 411-417.
- Johnson CM, Ulrich A (1950) Determination of nitrate in plant material. *Anal Chem* 22: 1526-1529.
- Katterer T, Reichstein M, Andren O, Lomander A (1998) Temperature dependence of organic matter decomposition: A critical review using literature data analysed with different models. *Biol Fertil Soils* 27: 258-262.
- Kenney DR, Nelson DW (1982) Nitrogen-Inorganic forms. In: *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*, 2nd ed. (Eds. AL Page, RH Miller, DR Kenney), American Society of Agronomy. Madison, Wisconsin, USA, pp. 711-730.
- Palm CA, Sanchez PA (1991) Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biol Biochem* 23: 83-88.
- Pang XP, Letey J (2000) Organic farming: Challenge of timing nitrogen availability to crop nitrogen requirements. *Soil Sci Soc Am J* 64: 247-253.
- Pansu M, Thuries L (2003) Kinetics of C and N mineralization, N immobilization and N volatilization of organic inputs in soil. *Soil Biology & Biochemistry* 35: 37-48.
- Poovatom S (1988) Nitrogen mineralization rate of the acidic, xeric soils of the New Jersey pinelands: Laboratory studies. *Soil Sci* 145: 337-343.
- Porto ML, Alves JC, De Souza AP, Araujo RC, Arruda JA (2008) Nitrate production and accumulation in lettuce as affected by mineral nitrogen supply and organic fertilization. *Horticultura Brasileira* 26: 227-230.
- Quemada M, Cabrera ML (1995) Carbon and nitrogen mineralized from leaves and stems of four cover crops. *Soil Sci Soc Am J* 59: 471-477.
- Safaa AM, Fattah AEMS (2007) Effect of nitrogen forms on nitrate contents and mineral composition in lettuce plants in sandy and calcareous soils. *Journal of Applied Sciences Research* 3: 1630-1636.
- Sierra J (1997) Temperature and soil moisture dependence of N mineralization in intact soil cores. *Soil Biol Biochem* 29: 1557-1563.
- Simakov VN, Tsyplenkov VP (1969) Procedures for the simultaneous determination of carbon, nitrogen, and oxidation degree in soil. *Agrokimiya* 6: 127-134.
- Soil Survey Staff (1993) Soil Survey Manual. USDA Handbook No. 18, Washington, USA.
- Sorensen JN, Johansen AS, Poulsen N (1994) Influence of growth and conditions on the value of crisphead lettuce: I. Marketable and nutritional quality as affected by nitrogen supply cultivar and plant age. *Plant Foods for Human Nutrition* 46: 1-11.
- Stanford G, Smith SJ (1972) Nitrogen mineralization potentials of soils. *Soil Sci Soc Amer Proc* 36: 465-472.
- Terbe I, Zsoldos L, Patócs I (1986) A zöldségnövények nitráttartalma. Lippay János Tudományos Ülésszak Előadásai 1: 125-131. (In Hungarian)
- Trinsoutrot S, Recous B, Bentz M, Lineres D, Cheneby D, Nicolardot B (2000) Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Sci Soc Am J* 64: 918-926.
- Tu C, Louws FJ, Creamer NG, Mueller JP, Brownie C, Fager K, Bell M, Hu S (2006) Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, Ecosystems and Environment* 113: 206-215.
- Vigil MF, Kissel DE (1995) Rate of nitrogen mineralized from incorporated crop residues as influenced by temperature. *Soil Sci Soc Am J* 59: 1636-1644.
- Wehrmann J, Scharpf HC (1979) Der Mineralstickstoffgehalt des Bodens als Massstab für den Stickstoffdüngungsbedarf (Nmin-Methode). *Plant Soil* 52: 109-126.
- Willer H, Yussefi M (2006) *The World of Organic Agriculture - Statistics and Emerging Trends 2006*. International Federation of Organic Agriculture Movements (IFOAM), Bonn, Germany and Research Institute of Organic Agriculture FiBL, Frick, Switzerland.