



# Nitrogen Mineralization from Organic Amendments Predicted by Laboratory and Field Incubations

Rui Pinto, Luís Miguel Brito & João Coutinho

To cite this article: Rui Pinto, Luís Miguel Brito & João Coutinho (2020) Nitrogen Mineralization from Organic Amendments Predicted by Laboratory and Field Incubations, Communications in Soil Science and Plant Analysis, 51:4, 515-526, DOI: [10.1080/00103624.2020.1717510](https://doi.org/10.1080/00103624.2020.1717510)

To link to this article: <https://doi.org/10.1080/00103624.2020.1717510>



Published online: 30 Jan 2020.



Submit your article to this journal [↗](#)



Article views: 35



View related articles [↗](#)



View Crossmark data [↗](#)



# Nitrogen Mineralization from Organic Amendments Predicted by Laboratory and Field Incubations

Rui Pinto<sup>a</sup>, Luís Miguel Brito <sup>b</sup>, and João Coutinho <sup>c</sup>

<sup>a</sup>C. Química Vila Real, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal; <sup>b</sup>Centro De Investigação De Montanha (CI MO), Escola Superior Agrária, Instituto Politécnico De Viana Do Castelo, Ponte De Lima, Portugal; <sup>c</sup>C. Química Vila Real, DeBA, EC Vida E Ambiente, Universidade De Trás-os-Montes E Alto Douro, Vila Real, Portugal

## ABSTRACT

Reliable methods to estimate nitrogen (N) mineralization from organic amendments are needed to improve the fertilization recommendations for organic horticultural crops. With this aim, N mineralization from farm-yard manure compost (FYMC), commercial organic fertilizer (COF) based on materials that are prompt to rapid N mineralization and green manure was determined in field situation where environmental factors affect mineralization rates and under controlled laboratory conditions that may not reflect conditions in the field. The ability of field and laboratory incubations to predict N mineralization was estimated during a field organic crop rotation set up with a cover crop of rye and vetch, followed by Portuguese cabbage and carrot. The relationship between mineralized N in field incubation and crop N accumulation suggests that field incubation is effective to predict N mineralization from FYMC, COF and green manure. However, the pattern of N mineralization from FYMC and green manure was different in field and laboratory incubation conditions as opposed to N mineralization from COF that was similar in both incubations.

## ARTICLE HISTORY

Received 2 July 2018

Accepted 13 January 2020

## KEYWORDS

Compost; green manure; organic agriculture; organic fertilizer

## Introduction

Organic farmers need information about organic fertilization practices such as green manuring and the use of composts and other organic amendments to sustain soil fertility and increase crop yields. Compost is the main source of organic matter (OM) and N supply for organic farming (Raviv et al. 2004) but frequently has low organic N content and a slow rate of mineralization (Cordovil et al. 2005). Nevertheless, the composting process yields a sanitized and stabilized organic material containing humic substances that contribute to the long-term mineralization, acting as a slow-release source of N (Hartl and Erhart 2005). In contrast to the small amount of mineralized N from mature composts in the short-term, commercial organic fertilizers which have higher contents of total N and labile N show larger available N contents and are often recommended in situations of high crop N demand (Brito et al. 2016). Green manures are cover crops incorporated into the soil that may contribute to the fertilization of the subsequent crop through the mineralization of the cover crop biomass (Salmeron, Isla, and Caverro 2011). After incorporation of fresh crops into the soil as green manure, soil microbial biomass increase because of the incorporation of easily degradable materials (Elfstrand, Bath, and Martensson 2007) and may lead to an increase in soil fertility (Kautz, Wirth, and Ellmer 2004). The enhancement of microbial activity also can promote the N mineralization of indigenous soil organic matter by the priming effect (Zhou et al. 2012).

Composts and green manures increase the soil organic matter pool of the soil. However, there is a need to predict the amount of mineralized N and the pattern of N release from the organic

amendments incorporated into the soil, to find the optimal rate and best time for fertilizer application to match nutrient supply with plant demand (Brito 2001).

Methods to estimate N mineralization from organic amendments using aerobic incubation under controlled temperature and water conditions in the laboratory are the most widely used (Zinati, Christenson, and Harris 2007). However, conflicting results have been reported because of changes of physical and chemical characteristics of the samples from sample perturbations during storing, mixing, and sieving (Arnold, Corre, and Veldkamp 2008; Makarov et al. 2017; Risch et al. 2019). In addition to sample manipulation, temperature and soil water conditions are different in the field than in laboratory incubation (Hanselman, Graetz, and Obreza 2004; Lomander, Katterer, and Andren 1998). Field incubations including polyethylene bags (Eno 1960), PVC cores (Raison, Connell, and Khanna 1987), or the addition of acetylene to prevent N losses through denitrification (Hatch, Jarvis, and Philips 1990) were used to estimate N mineralization under temperature and moisture conditions that exist in undisturbed soil. Although field incubations showed limitations associated with disturbance of soil and isolation of the soil from natural drying-wetting cycles during sampling and incubation (Hatch, Jarvis, and Philips 1990), the buried bag technique prevents leaching losses and provides an accurate estimation of N mineralization. It is also helpful to validate the N mineralization models obtained from laboratory studies (Monaco et al. 2010; Sierra 1997; Wienhold 2007).

Nitrogen mineralization from organic amendments determined in laboratory and field incubations are not consistent because of differences in the applied methodology and environmental conditions (Arnold, Corre, and Veldkamp 2008; Hanselman, Graetz, and Obreza 2004). Laboratory methods are difficult to extrapolate to field conditions because the amount of mineralized N depends on the method used to handle and to store the samples and the models need to be calibrated on site (Benbi and Richter 2002). In this context, information about N mineralization dynamics in the field is crucial to determine the amount of N that is available to the crops, and to improve fertilizer recommendations for organic farming. We hypothesized that N mineralization from organic amendments whose chemical characteristics promote N mineralization (low C/N ratio and high amounts of soluble C and N), can be estimated by laboratory incubation. On the contrary, we hypothesized that N mineralization from the organic amendments whose chemical characteristics do not promote N mineralization would be difficult to predict under controlled laboratory conditions because their mineralization depends heavily on biological community. Therefore, the objectives of this study were: (i) to estimate N availability from farmyard manure compost (FYMC), immature commercial organic fertilizer (COF), and rye consociated with vetch as green manure according to their chemical characteristics in field and laboratory incubations; and (ii) to assess the ability of these incubations to predict the pattern of N release from these organic amendments during a field organic crop rotation.

## Materials and methods

### Study site description

The field experiment was carried out in a sandy loamy Dystric Cambisol (IUSS, 2006) located at the NW of Portugal, in Fafe (41° 12' N and 8° 20' W), approximately at 300 m altitude. The soil was of granite origin with 784 g kg<sup>-1</sup> sand, 163 g kg<sup>-1</sup> lime and 52 g kg<sup>-1</sup> clay and a bulk density of 1.04. The soil reaction was acidic with a pH (H<sub>2</sub>O) value of 4.9; consequently, dolomite lime was applied at 5 t ha<sup>-1</sup> of CaCO<sub>3</sub> equivalent. The soil electrical conductivity was 0.03 dS m<sup>-1</sup>; the total N content was 1.9 g kg<sup>-1</sup>, and the soil organic C content was 29 g kg<sup>-1</sup>.

The experiment was set up in 2014 with a cover crop of rye (*Secale cereale* L.) consociated with vetch (*Vicia sativa* L. cv. Barril) grown over the autumn and winter, for green manure, followed by Portuguese cabbage (*Brassica oleracea* var. tronchuda cv. Penca da Póvoa) from April to June and carrot (*Dacus carota* L. cv. Jarana F1) from June to October. The horticultural crops were irrigated

with a system of mini-sprinklers ( $200 \text{ Lh}^{-1}$ ) placed at a distance of 6.5 m between them so that the water content in the soil was not limiting for plant growth. Hoe blade weeding was carried out frequently to avoid weed competition.

Soil and air temperature were registered automatically with two thermistors, one buried in the soil at 10cm depth and another below a reflector board at 30cm height. The temperature was registered every hour with a DL2 Data Logger (Delta-T Devices, Cambridge, United Kingdom) and the average daily air and soil temperatures were calculated.

### Experimental treatments

The experimental crop rotation was set up as a randomized complete block design with four blocks to quantify crop N accumulation and N mineralization determined by laboratory and field incubations. The experimental site with  $240 \text{ m}^2$  was divided in 16 plots of  $15 \text{ m}^2$  each. The treatments included: (i)  $22 \text{ t ha}^{-1}$  green manure (GM); (ii) GM with  $40 \text{ t ha}^{-1}$  FYMC (C40); (iii) GM with  $2 \text{ t ha}^{-1}$  COF (CF2) and (iv) a reference treatment without fertilizers (T0). The FYMC, COF, and green manure characteristics are shown in Table 1.

### Farmyard manure compost

Composting piles were prepared outdoor with cow manure and wheat straw as bedding material accumulated for one year in the cattle shed. The feedstock was composted for approximately 7 months and manually turned twice at 2 and 4 months after the start of composting. The FYMC was matured as indicated by the concentration of  $\text{NH}_4^+\text{-N}$  ( $81 \text{ mg kg}^{-1}$ ) below the maximum recommended value ( $400 \text{ mg kg}^{-1}$  DM) for mature composts by Zucconi and de Bertoldi (1987) and by the rate  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{N}$  (0.06) which was below to the suggested upper limit value of 1 for matured composts by Larney and Hao (2007).

### Commercial organic fertilizer

The commercial organic fertilizer traded by (Crimolara, Lisboa, Portugal) certified for organic agriculture was based on granulated poultry manure, beet molasses and feathers. In contrast, the COF was not matured as indicated by the concentration of  $\text{NH}_4^+\text{-N}$  ( $5309 \text{ mg kg}^{-1}$ ) far above the maximum recommended value ( $400 \text{ mg kg}^{-1}$  DM).

### Field incubation

The field incubation was set up as a plant growing experiment during the Spring-Summer growing season immediately after the incorporation of the organic amendments previously to planting the

**Table 1.** Dry matter content (DM) and chemical characteristics of farmyard manure compost (FYMC), commercial organic fertilizer (COF), or green manure (mean, standard deviation).

Characteristic	Unit	FYMC	COF	Green manure
DM	(%)	24.7	90.5	20.1
pH		$7.9 \pm 0.02$	$6.9 \pm 0.01$	
EC	( $\text{dS m}^{-1}$ )	$5.2 \pm 0.8$	$6.9 \pm 0.2$	
C	( $\text{g kg}^{-1}$ )	$313 \pm 37$	$423 \pm 5$	$399 \pm 22$
N total	( $\text{g kg}^{-1}$ )	$25.4 \pm 1.5$	$99.6 \pm 5.0$	$20.6 \pm 1.7$
C/N		$12 \pm 1.7$	$4.3 \pm 0.2$	$20 \pm 3.0$
Soluble C	( $\text{g kg}^{-1}$ )	$7.8 \pm 0.5$	$52.2 \pm 1.3$	$70.4 \pm 1.5$
Soluble N	( $\text{g kg}^{-1}$ )	$1.2 \pm 0.2$	$21.2 \pm 0.3$	$6.1 \pm 0.2$
$\text{NH}_4^+\text{-N}$	( $\text{mg kg}^{-1}$ )	$81 \pm 9$	$5309 \pm 150$	
$\text{NO}_3^-\text{N}$	( $\text{mg kg}^{-1}$ )	$1334 \pm 30$	$135 \pm 9$	
P	( $\text{g kg}^{-1}$ )	$5.8 \pm 0.3$	$12.8 \pm 0.6$	$3.9 \pm 1.1$
K	( $\text{g kg}^{-1}$ )	$29 \pm 2.2$	$25.8 \pm 9.5$	$23.6 \pm 3.6$

Nutrient concentrations are expressed on a dry matter basis

Portuguese cabbage, based on the methodology described by Raison, Connell, and Khanna (1987). The soil samples were collected every 14 days during the incubation experiment except for the first period that was of 7 days. Simultaneously, four plants from each replicate were harvested for analysis to quantify crop total N content. At the start of each incubation period, five pairs of samples were collected randomly from each plot using PVC cores to avoid soil disturbance. The cores had a length of 15 cm, 4 cm diameter, and six holes with 6 mm of diameter to allow aeration. One sample from each pair was frozen, and the remaining five samples were enclosed in micro-perforated polyethylene bags and buried at 20 cm depth. Net N mineralization was calculated by the difference between the amount of inorganic N ( $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{N}$ ) in incubated and frozen samples. The accumulated mineralized N during incubation was calculated by the sum of N mineralized during each period of incubation and provided the estimate of total mineralized N in the tilled layer. Nitrogen mineralized from the FYMC and COF ( $\text{mg kg}^{-1}$  soil) was estimated by the difference between mineralized N ( $\text{mg kg}^{-1}$  soil) in treatments C40 and CF2 and mineralized N ( $\text{mg kg}^{-1}$  soil) in treatment GM. The green manure mineralized N was calculated by the difference between mineralized N ( $\text{mg kg}^{-1}$  soil) in treatments GM and T0. Nitrogen mineralization ( $\text{g kg}^{-1}$  initial N) from these soil amendments was calculated by the ratio between accumulated mineralized N ( $\text{mg kg}^{-1}$  soil) from FYMC, COF or green manure during the incubation period and the total amount of  $\text{N}_{\text{org}}$  applied ( $\text{g kg}^{-1}$  soil) (equation 1).

$$\text{Nm} = \sum_{i=1}^{13} \text{Nti} / \text{N}_{\text{org}} \quad (1)$$

Where Nm is N mineralization from soil amendments ( $\text{g kg}^{-1}$  initial N); Nti is mineralized N during each period (i) of incubation ( $\text{mg kg}^{-1}$  soil);  $\text{N}_{\text{org}}$  is the total amount of organic N applied ( $\text{g kg}^{-1}$  soil).

### Laboratory incubation

The soil was pre-incubated at 25°C for 15 days, in the dark, prior to laboratory incubation procedures. The laboratory incubation was set up with samples of 60 g of soil (dry weight basis) prepared according to the experimental treatments incorporated into the soil in the field experiment. The fertilized treatments included: (i) FYMC; (ii) COF; (iii) green manure; (iv) FYMC with green manure; (v) COF with green manure and (vi) a reference treatment. The FYMC was applied at 7.5 g DM  $\text{kg}^{-1}$ ; the COF was applied at the rate of 1.4 g DM  $\text{kg}^{-1}$  and green manure was applied at the rate of 3.3 g  $\text{kg}^{-1}$ . Before the experiment, soil moisture was adjusted to 70% of soil water-holding capacity by adding water gradually and then thoroughly stir the mixture.

The soil samples of each treatment (4 replications) were placed in plastic containers and put into 1-L glass jars. These subsamples were removed from the glass jars for N mineral analyzes after 1, 3, 7, 10, 14, 21, 35, 56, 84, 112 and 168 days and replaced by the subsamples stored in plastic containers in the beginning of the incubation. The plastic containers and the jars sealed with air-tight glass lids were incubated for 168 days at 25°C. The plastic containers were weighted every 15 days to adjust water content. The glass jars contained a vessel with 20 ml of NaOH (1M) solution to trap evolved  $\text{CO}_2$  and a vessel with 40 ml of distilled water to avoid desiccation. After 1, 2, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 70, 84, 98, 112, 126, 140, 154, 168 days, the vessels with NaOH solution were removed, and the trapped  $\text{CO}_2$  was determined with an elemental analyzer with near-infrared detector (NIRD) Formacs<sup>HT</sup> Analyzer (Skalar, Breda, The Netherlands) and replaced with fresh NaOH (Gardner et al. 2013). The apparent loss of added organic materials C was estimated by subtracting total  $\text{CO}_2\text{-C}$  released in a given treatment from that measured in the reference treatment. The accumulated mineralized N was calculated for 168 days (crop growth period).

### Analytical methods

The horticultural crop sampling was based on four plants of each replicate treatment. Crop samples were dried at 65°C until constant weight, milled using a 2-mm sieve and subsequently

used to determine total N content. The FYMC, COF, and green manure samples were frozen and, subsequently, freeze-dried to reduce water content. Dry matter content (DM), pH, and electrical conductivity (EC) were determined by standard procedures (CEN, 1999). Total C was determined by near-infrared detector in an elemental analyzer (Primacs<sup>SNC-100</sup> Analyzer, Skalar, Breda, The Netherlands) after combustion at 950°C (Temminghoff and Houba 2004). Total N and P was measured by molecular spectrophotometry (San<sup>Plus</sup> System, Skalar, Breda, The Netherlands) and K was quantified by flame photometry (PFP7 Flame Photometer, Jenway, Stadfforshire, UK) after digestion with sulfuric acid (Temminghoff and Houba 2004). Mineral N was extracted from 6 g fresh samples of soil, FYMC and COF samples using KCl 1:5 solution ratio and NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents were determined by molecular absorption spectrophotometry with a segmented flow analyzer system (San<sup>Plus</sup> System, Skalar, Breda, The Netherlands) (Houba et al. 1989) For green manure, FYMC, and COF, soluble C and N were extracted from 4 g dry samples with 0.01 M CaCl<sub>2</sub> at a ratio of 1:20 and determined by near-infrared detector and chemiluminescence, respectively, in an elemental analyzer (Formacs<sup>HT</sup> Analyzer, Skalar, Breda, The Netherlands) after combustion at 950°C (Boon et al. 2014).

### **Statistical analysis and kinetic model**

The first-order kinetic model with one mineralization pool (Bonde and Lindberg 1988) was fitted for both incubations to N mineralization from FYMC, COF, and green manure (equation 2).

$$Nm = N_0 [1 - \exp(-k_1 t - k_2 t^2)] \quad (2)$$

Where Nm is estimated N mineralization (g kg<sup>-1</sup> of initial N) at time t (days after incorporation of FYMC, COF or green manure); k<sub>1</sub> and k<sub>2</sub> are mineralization constant rates (the quotient -k<sub>1</sub>/k<sub>2</sub> defines the immobilization time period); and the amount of potentially organic mineralizable N pool is given by N<sub>0</sub> (g kg<sup>-1</sup> of initial N).

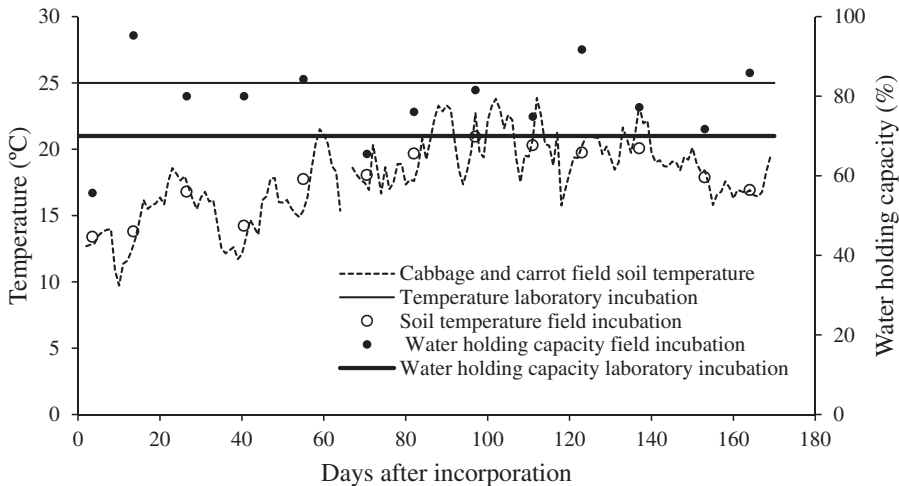
Model equations were fitted by the non-linear least-square curve-fitting technique (Marquardt-Levenberg algorithm), to minimize the sum of the squared differences between the observed and predicted values of the dependent variable. Analyses of variance (ANOVA) were performed to calculate equations F values to test the dependence of mineralization on incubation time. The Pearson correlation coefficient was used to validate the equations and to assess the strength of the linear association between N accumulated by the crops and the accumulated mineralized N. All statistical calculations were performed using SPSS v. 17.0 for windows (SPSS Inc. Chicago, USA) (Suárez et al. 2011).

## **Results and discussion**

### **Nitrogen mineralization**

Nitrogen mineralization depends on the activity of microorganisms, which is closely associated to temperature and soil water. Soil temperature in the laboratory was constant (25°C) whereas, soil daily temperature in the field fluctuated from 9.7°C to 23.9°C during the growth period of the vegetable crops. The field average soil temperature during the growth period of cabbage (from 22<sup>nd</sup> April to 17<sup>th</sup> June) and carrot (from 22<sup>nd</sup> June to 1<sup>st</sup> October) was 15.4°C and 19.4°C, respectively. Soil water content in laboratory incubation was approximately constant (70% water holding capacity), while the overall average soil water holding capacity (WHC) during the growth period of cabbage and carrot was 78%. The minimum and maximum WHC ranged between 56% and 95% in the field incubation (Figure 1).

Nitrogen mineralization was expected to increase in warmer laboratory conditions compared to field incubation (Wang et al. 2006), but this did not happen for FYMC, COF and, green manure (Table 2). Actually, soil biological activity promotes N mineralization over a large range of soil temperature and soil water contents (Borowik and Wyzkowska 2016a, 2016b). The maximum



**Figure 1.** Soil daily mean temperature during the growth period of cabbage and carrot, average soil temperature and water holding capacity (WHC, %) for each incubation period in field incubation, temperature and WHC (%) in laboratory incubation.

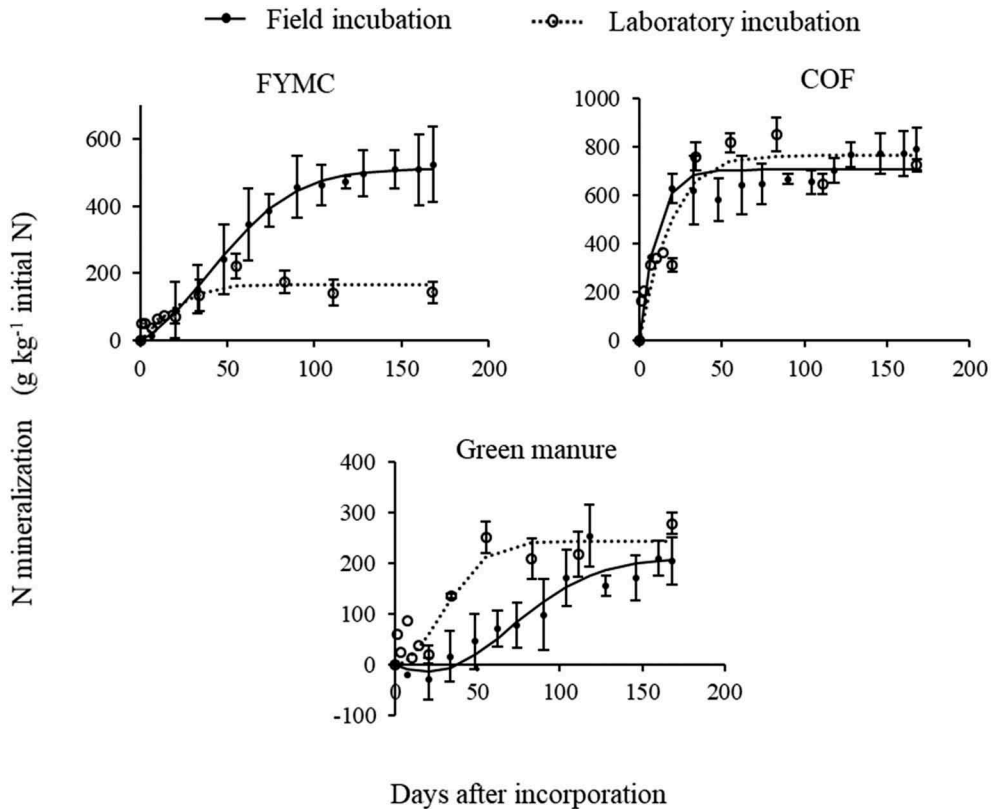
**Table 2.** Nitrogen mineralization ( $\text{g kg}^{-1}$  of initial N) equations, F values and determination coefficients ( $R^2$ );  $N_{\text{org}}$  applied ( $\text{g kg}^{-1}$  soil) and estimated N mineralization ( $\text{g kg}^{-1}$  initial N) during 168 days, for farmyard manure compost (FYMC), commercial organic fertilizer (COF) and green manure during field and laboratory incubation. \*\*  $p < .01$  \*\*\*  $p < .001$ .

Organic amendments	Model equation	$N_{\text{org}}$ applied ( $\text{g kg}^{-1}$ soil)	N mineralization ( $\text{g kg}^{-1}$ initial N)
Field incubation			
FYMC	$Nm = 510 [1 - \exp(-0.005t - 0.000199t^2)]$ $F_{(3,11)} = 5181^{***}$ $R^2 = 0.99^{***}$	237	508
COF	$Nm = 705 [1 - \exp(-0.094t - 0.000336t^2)]$ $F_{(3,11)} = 461^{***}$ $R^2 = 0.92^{***}$	171	705
Green manure	$Nm = 210 [1 - \exp(0.007t - 0.000187t^2)]$ $F_{(3,11)} = 88^{***}$ $R^2 = 0.91^{***}$	91	203
Laboratory incubation			
FYMC	$Nm = 167 [1 - \exp(-0.034t - 0.000555t^2)]$ $F_{(3,9)} = 51^{***}$ $R^2 = 0.81^{**}$	237	167
COF	$Nm = 764 [1 - \exp(-0.049t - 0.000264t^2)]$ $F_{(3,9)} = 89^{***}$ $R^2 = 0.88^{***}$	171	764
Green manure	$Nm = 243 [1 - \exp(-0.001t - 0.000657t^2)]$ $F_{(3,9)} = 43^{***}$ $R^2 = 0.86^{***}$	91	243

aerobic microbial activity occurs between 50 and 70% WHC (Stres et al. 2008). However, Agehara and Warncke (2005) reported that N mineralization during 12 weeks of incubation was not statistically different between 70% and 90% WHC. These authors also found that N mineralization was similar between 15–20°C and 20–25°C. In fact, soil microbial community can adapt to low temperatures (Borowik and Wyszowska 2016a). Soil microbial community is likely controlled through feedback of enzyme activity, favoring the microbes that produce enzymes with increased activity in field conditions (Wallenstein et al. 2011). Moreover, several authors found that soil wetting and drying cycles may increase N mineralization (Rey et al. 2005; Xiang et al. 2008). Probably for the reason that drying provides new substrates from dead microbial biomass (Mengel et al. 1996). Also, the mechanical disruption of soil aggregates caused by drying and rewetting may increase N mineralization (Smith et al. 2003).

The mineralized N from green manure at the end of the experimental period (168 days) was comparable under field or laboratory incubations. However, the pattern of N release was different between field incubation and controlled laboratory conditions since N mineralization from green





**Figure 2.** N mineralization ( $\text{g kg}^{-1}$  initial N) for farmyard manure compost (FYMC), commercial organic fertilizer (COF) or green manure during field and laboratory incubations. N mineralization equations are described in Table 2.

manure decreased during the first days of field incubation compared to laboratory incubation (Figure 2). This decrease can be explained by the increase in soil water content to full WHC from 7 to 20 days after the beginning of field incubation (Figure 1) that may have induced a negative effect on N mineralization (Hassan 2013; Murwira, Kirchmann, and Swift 1990). The highest C/N ratio in green manure (20) compared to FYMC (12) and COF (4) promoted a slight N immobilization (38 days) as the turning point between N mineralization and immobilization ranges between 15 and 40 (Cabrera, Kissel, and Vigil 2005; Salmeron, Isla, and Caverio 2011).

The N mineralized from FYMC was slightly lower in field incubation than in laboratory incubation between 7 to 20 days probably due to the increase of soil water content similar to what happened after green manure incorporation. However, after 168 days of incubation, N mineralization from the FYMC clearly increased from  $167 \text{ g kg}^{-1}$  initial N in laboratory conditions to  $508 \text{ g kg}^{-1}$  of initial N in the field incubation (Table 2). Although smaller for laboratory conditions compared to field incubation, the FYMC N mineralization in laboratory incubation ( $167 \text{ g kg}^{-1}$  initial N) was within the range of values commonly found in laboratory incubations (Antil et al. 2011; Hadas and Portnoy 1997; Morvan and Nicolardot 2009).

In laboratory incubation,  $\text{CO}_2\text{-C}$  evolved from green manure ( $955 \text{ mg kg}^{-1}$ ) was much greater than the amount of  $\text{CO}_2\text{-C}$  evolved from FYMC ( $78 \text{ mg kg}^{-1}$ ) indicating an increase in soil microbial activity with green manure (Table 3). The  $\text{CO}_2\text{-C}$  evolved from FYMC was 1.5 times the soluble C and approximately 4% of total C. Probably, the addition of available C from the fresh cover crops into the soil contributed to enhance available C and the activity of soil microorganisms



**Table 3.** Applied C ( $\text{mg kg soil}^{-1}$ ) and evolved  $\text{CO}_2\text{-C}$  ( $\text{mg kg soil}^{-1}$ ) from farmyard manure compost (FYMC), commercial organic fertilizer (COF), green manure (GM), FYMC with GM and COF with GM, in the laboratory incubation.

Organic amendments	C applied, $\text{mg kg}^{-1}$		$\text{CO}_2\text{-C}$ evolved, $\text{mg kg}^{-1}$
	Total C	Soluble C	
FYMC	1982	49	78
COF	491	61	236
Green manure	1136	200	955
FYMC + green manure	3118	249	1066
COF + green manure	1627	261	1113

(Elfstrand, Bath, and Martensson 2007; Kautz, Wirth, and Ellmer 2004) and probably increased N mineralization from FYMC in the field incubation.

The decreasing of N mineralization from FYMC in laboratory incubation may be related to a significant change and decrease of microbial biomass due to freezing of the samples before laboratory incubation. For example, Wallenius et al. (2010) found a decrease between 20% and 30% of enzyme activity in compost after freezing. Moreover, frozen storage reduced accumulated  $\text{CO}_2$  evolution in composts by 110 to 277% depending on the maturation degree of the compost, indicating a deep decrease of microbial activity (Wu and Ma 2001). On the other hand, this increase of growth and activity of soil microorganisms can promote the degradation of recalcitrant soil organic matter (priming effect), increasing N mineralization in the field (Blagodatskaya and Kuzyakov 2008; Fu et al. 2000; Pascault et al. 2013). In addition, materials derived from root exudates that may increase N mineralization are excluded in laboratory incubation (Yamasaky, Taterno, and Shibata 2011).

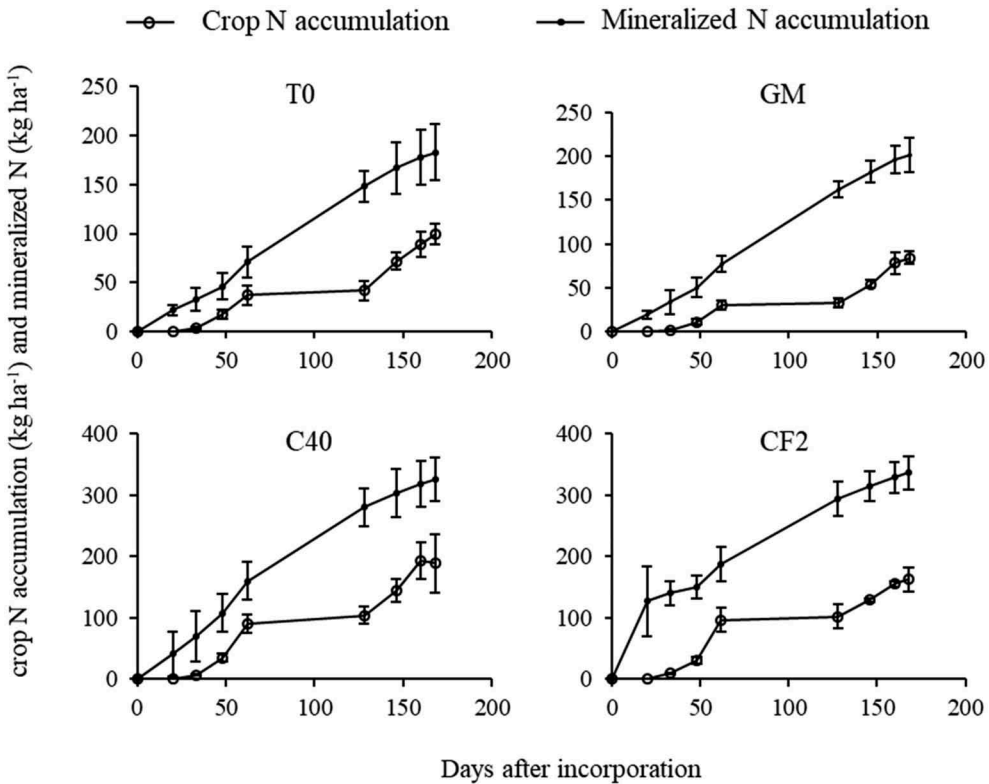
The pattern of mineralized N from COF was similar for field and controlled laboratory conditions (Figure 2). The COF was rapidly mineralized in both types of incubation and the mineralized N ( $N_m$ ), predicted by the mineralization model was 705 and 764  $\text{g kg}^{-1}$  of initial N for field and laboratory incubations, respectively (Table 2). The mineralized N from COF increased fast even under less favorable climatic conditions (high soil moisture content). Eldridge et al. (2008) also concluded that the accumulated mineralized N from immature biosolid fertilizer ( $C/N = 6$ ) was very similar in field and laboratory incubations (611 and 595  $\text{kg ha}^{-1}$ , respectively).

In contrast to FYMC, the amount of  $\text{CO}_2\text{-C}$  evolved from COF during the laboratory incubation period was approximately 4 times the amount of COF soluble C, and approximately half the amount of its total C (Table 3). Hence, the chemical characteristics of COF, independently of the presence of green manure led to rapid N mineralization after quick colonization by the soil microorganisms. Indeed, high total N (99.6  $\text{g kg}^{-1}$ ) and mineral N contents (5.4  $\text{g kg}^{-1}$ ), low C/N ratio (4.3), and high amount of available C and N (52.2 and 21.2  $\text{g kg}^{-1}$ , respectively) were also reported by other authors to increase microbial activity with the release of high amounts of mineral N in a short period of time (Antil et al. 2011; Martin-Olmedo and Rees 1990; Stadler et al. 2006).

### Crop nitrogen uptake

Crop N accumulation ( $\text{kg ha}^{-1}$ ) was calculated multiplying crop total N content ( $\text{g kg}^{-1}$ ) by crop dry weight ( $\text{t ha}^{-1}$ ). Crop N uptake is usually less than 50% of soil available mineral N (Fageria and Baligar 2005). Here, N accumulation for both crops ranged from 42% to 58% of mineralized N in the 0–15 cm top layer of soil, during field incubation. These values are comparable to those reported by Carsky et al. (1990) who found that N accumulation in the above ground of maize was 69% of N mineralization in the 0–15 cm top layer determined by buried bag incubation technique.

The FYMC released 120.4  $\text{kg N ha}^{-1}$  of available N ( $237 \text{ kg ha}^{-1} \times 0,508 \text{ g kg}^{-1}$  initial N) for the growth period of cabbage and carrot (Table 2). As the increase in N accumulation for both crops was



**Figure 3.** Crop N accumulation in cabbage and carrot and accumulated mineralized N in the field incubation for green manure (GM), GM with FYMC (C40), GM with COF (CF2) and for the control treatment without fertilization (T0).

103.8 kg ha<sup>-1</sup> with FYMC (difference between crop N accumulation in C40 and GM treatments) it may be suggested that these crops recovered 86% of the mineralized N from FYMC (Figure 3). Although the available N released from COF (120.2 kg ha<sup>-1</sup>) was similar to FYMC, crop N accumulation increased only 78.0 kg ha<sup>-1</sup>. Therefore, N recovery by the crops for this organic fertilizer decreased to 65%, which means that one-third of available N would probably be leached after COF application, whereas only 14% of FYMC mineralized N was prone to leaching.

In the laboratory incubation, the N release from FYMC during 168 days (39.6 kg ha<sup>-1</sup>) was much below the increase on crop N accumulation (103.8 kg ha<sup>-1</sup>) with FYMC application. Therefore, laboratory incubation was not effective to predict N mineralization from FYMC. On the contrary, N recovery by the crops from COF was similar under laboratory and field conditions (60 and 65% respectively), confirming that both incubations may have accurately predict N mineralization from COF. In addition, a strong positive correlation ( $p < .001$ ) between mineralized N and crop N accumulation during the growth period of both crops was found in the field incubation for T0, GM, C40 and CF2 ( $R^2 = 0.89$ ;  $R^2 = 0.88$ ;  $R^2 = 0.91$  and  $R^2 = 0.83$  respectively). Therefore, the field incubation may be a useful indicator to predict N availability from different organic amendments, with more or less rapid mineralization, as suggested by Yan et al. (2006) and Monaco et al. (2010).

## Conclusions

Nitrogen mineralization from COF with high total N content, low C/N ratio and high amounts of available C and N was accurately predicted by both, laboratory and field incubations. On the

contrary, N mineralization from FYMC and green manure were significantly different in field incubation compared to laboratory conditions. Therefore, more reliable laboratorial methods will be needed to improve the similarity between N mineralization under laboratory and field conditions.

The strong correlation found between mineralized N in field incubation and crop N accumulation indicates that field incubation can be a reliable method to estimate N availability for crop uptake.

## Funding

This work was supported by the Fundação para a Ciência e a Tecnologia [grant number SFHR/BD/77447/2011];

## ORCID

Luís Miguel Brito  <http://orcid.org/0000-0002-6606-2963>

João Coutinho  <http://orcid.org/0000-0002-6303-9549>

## References

- Agehara, S., and D. D. Warncke. 2005. Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. *Soil Science Society of America Journal* 69:1844–55. doi:10.2136/sssaj2004.0361.
- Antil, R. S., A. Bar-Tal, P. Fine, and A. Hadas. 2011. Predicting nitrogen and carbon mineralization of composted manure and sewage sludge in soil. *Compost Science and Utilization* 19:33–43. doi:10.1080/1065657X2011-10736974.
- Arnold, J., M. D. Corre, and E. Veldkamp. 2008. Cold storage and laboratory incubation of intact soil cores do not reflect in-situ nitrogen cycling rates of tropical forest soils. *Soil Biology & Biochemistry* 40:2480–83. doi:10.1016/soilbio.2008.06.001.
- Benbi, K. D., and J. Richter. 2002. A critical review of some approaches to modelling nitrogen mineralization. *Biology and Fertility of Soils* 35:168–83. doi:10.1007/s00374-002-0456-6.
- Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils* 45:115–31. doi:10.1007/s00374-008-0334-y.
- Bonde, T. A., and T. Lindberg. 1988. N mineralization kinetics in soil during long-term aerobic laboratory incubation: A case study. *Journal of Environmental Quality* 17:414–17. doi:10.2134/jeq1988.00472425001700030011.
- Boon, A., J. S. Robinson, D. R. Chadwick, and L. M. Cardens. 2014. Effect of cattle urine addition on the surface emissions and subsurface concentrations of greenhouse gases in a UK peat grassland. *Agriculture, Ecosystems and Environment* 186:23–32. doi:10.1016/j.agee.2014.01.008.
- Borowik, A., and J. Wyszowska. 2016a. Impact of temperature on the biological properties of soil. *International Agrophysics* 30:1–8. doi:10.1515/intag-2015-0070.
- Borowik, A., and J. Wyszowska. 2016b. Soil moisture as a factor affecting the microbiological and biochemical activity of soil. *Plant Soil and Environment* 62:250–55. doi:10.17221/158/2016-PSE.
- Brito, L. M. 2001. Lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea* L. var. capitata L.) growth in soil mixed with municipal solid waste compost and paper mill ludge composted with bark. *Acta Horticulturae* 563:131–37. doi:10.17660/ActaHortic.2001.563.16.
- Brito, L. M., A. Sampaio, R. Pinto, I. Mourão, and J. Coutinho. 2016. Lettuce response to organic and phosphate fertilizers and root mycorrhization. *Journal of Plant Nutrition* 39:842–49. doi:10.1080/01904167.2015.1106557.
- Cabrera, M. L., D. E. Kissel, and M. F. Vigil. 2005. Nitrogen mineralization from organic residues: Research opportunities. *Journal of Environmental Quality* 34:75–79. doi:10.2134/jeq2005.0075.
- Carsky, R. J., W. S. Reid, A. R. Suhet, and D. J. Lathwell. 1990. Screening legume green manures as nitrogen sources to succeeding non-legume crops. *Plant and Soil* 128:275–82. doi:10.1007/BF00011119.
- CEN, 1999. *European standards - soil improvers and growing media*. European Committee for Standardization
- Cordovil, C. M., J. Coutinho, M. Goss, and F. Cabral. 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. doi:10.1079/SUM2005294Eldridge.
- Eldridge, S. M., K. Y. Chan, Z. H. Xu, C. R. Chen, and I. Barchia. 2008. Plant available nitrogen supply from granulated biosolids: Implications for land application guidelines. *Australian Journal of Soil Research* 46:423–36. doi:10.1071/SR07234.
- Elfstrand, S., B. Bath, and A. Martensson. 2007. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied Soil Ecology* 36:70–82. doi:10.1016/j.apsoil.2006.11.001.

- Eno, C. F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. *Soil Science Society of America Journal* 24:277–79. doi:10.2136/sssaj1960.03615995002400040019x.
- Fageria, N. K., and V. C. Baligar. 2005. Enhancing nitrogen use efficiency in crop plants. *Advances in Agronomy* 88:97–185. doi:10.1016/S0065-2113(05)88004-6.
- Fu, S., D. C. Coleman, R. Schartz, R. Potter, P. F. Hendrix, and D. A. Crossley. 2000. <sup>14</sup>C distribution in soil organisms and respiration after the decomposition of crop residue in conventional tillage and no-till agroecosystems at Georgia Piedmont. *Soil Tillage Research* 57:31–41. doi:10.1016/S0167-1987(00)00133-1.
- Gardner, R. D., E. Lohman, R. Gerlach, K. E. Cooksey, and B. M. Peyton. 2013. Comparison of CO<sub>2</sub> and bicarbonate as inorganic carbon sources for triacylglycerol and starch accumulation in *Chlamydomonas reinhardtii*. *Biotechnology and Bioengineering* 110:87–96. doi:10.1002/bit.24592.
- Hadas, A., and R. Portnoy. 1997. Rates of decomposition in soil and release of available nitrogen from cattle manure and municipal wastes composts. *Compost Science & Utilization* 5:48–56. doi:10.1080/1065657X.1997.10701885.
- Hanselman, T. A., D. A. Graetz, and T. A. Obreza. 2004. A comparison of In Situ methods for measuring net nitrogen mineralization rates of organic soil amendments. *Journal of Environmental Quality* 33:1098–105. doi:10.2134/jeq2004.1098.
- Hartl, W., and E. Erhart. 2005. Crop nitrogen recovery and soil nitrogen dynamics in a 10-year field experiment with biowaste compost. *Journal of Plant Nutrition and Soil Science* 168:781–88. doi:10.1002/jpln.200521702.
- Hassan, W. 2013. C and N mineralization and dissolved organic matter potentials of two contrasting plant residues: Effects of residue type, moisture, and temperature. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science* 63:642–52. doi:10.1080/09064710.2013.846398.
- Hatch, D. J., S. C. Jarvis, and L. Philips. 1990. Field measurement of nitrogen mineralization using soil core incubation and acetylene inhibition of nitrification. *Plant and Soil* 124:97–107. doi:10.1007/BF00010937.
- Houba, V. J. G., J. J. Van der Lee, I. Novozamsky, and I. Walling. 1989. *Soil and PLANTANALYSIS, Part 5. Soil analysis procedures*. The Netherlands: Wageningen Agricultural University.
- IUSS (International Union of Soil Sciences) Working Group WRB. 2006. World reference base for soil resources. World Soil Resources Report n° 103. FAO, Rome.
- Kautz, T., S. Wirth, and F. Ellmer. 2004. Microbial activity in a sandy arable soil is governed by the fertilization regime. *European Journal of Soil Biology* 40:87–94. doi:10.1016/j.ejsobi.2004.10.001.
- Larney, F. J., and X. Hao. 2007. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta Canada. *Bioresource Technology* 98:3221–27. doi:10.1016/j.biortech.2006.07.005.
- Lomander, A., T. Katterer, and O. Andren. 1998. Modelling the effects of temperature and moisture on CO<sub>2</sub> evolution from top and subsoil using a multi-compartment approach. *Soil Biology & Biochemistry* 30:2023–30. doi:10.1016/S0038-0717(98)00077-7.
- Makarov, M. I., E. Y. Kuznetsova, T. I. Malysheva, M. N. Maslov, and O. V. Menyailo. 2017. Effect of the storage conditions of soil samples on carbon and nitrogen extractability. *Eurasian Soil Science* 50:549–58. doi:10.1134/S1064229317030085.
- Martin-Olmedo, P., and R. M. Rees. 1990. Short-term N availability in response to dissolved-organic-carbon from poultry manure, alone or in combination with cellulose. *Biology and Fertility of Soils* 29:386–93. doi:10.1007/s003740050569.
- Mengel, K. 1996. Turnover of organic nitrogen in soils and its availability to crops. *Plant and Soil* 181:83–93. doi:10.1007/BF00011295.
- Monaco, S., D. Sacco, T. Borda, and C. Grignani. 2010. Field measurement of net nitrogen mineralization of manured soil cropped to maize. *Biology and Fertility of Soils* 46:179–84. doi:10.1007/s00374-009-0412-9.
- Morvan, T., and B. Nicolardot. 2009. Role of organic fractions on C decomposition and N mineralization of animal wastes in soil. *Biology and Fertility of Soils* 45:477–86. doi:10.1007/s00374-009-0355-1.
- Murwira, H. K., H. Kirchmann, and M. J. Swift. 1990. The effect of moisture on the decomposition rate of cattle manure. *Plant and Soil* 122:197–99. doi:10.1007/BF02851975.
- Pascual, N., L. Ranjard, A. Kaisermann, D. Bachar, R. Christen, S. Terrat, O. Mathieu, J. Lévêque, C. Mougel, C. Henault, et al. 2013. Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems* 16:810–22. doi:10.1007/s10021-013-9650-7.
- Raison, R. J., M. J. Connell, and P. K. Khanna. 1987. Methodology for studying fluxes of soil mineral-N in situ. *Soil Biology & Biochemistry* 19:521–30. doi:10.1002/jpln.200520579.
- Raviv, M., S. Medina, A. Krasnovsky, and H. Ziadna. 2004. Organic matter and nitrogen conservation in manure compost for organic agriculture. *Compost & Science Utilization* 12:6–10. doi:10.1080/1065657X.2004.10702151.
- Rey, A., C. Petsikos, P. G. Jarvis, and J. Grace. 2005. Effect of temperature and moisture on rates of carbon mineralization in a mediterranean oak forest soil under controlled and field conditions. *European Journal Of Soil Science* 56:589–599. doi:10.1111/j.1365-2389.2004.00699x.
- Risch, A. C., S., R. Zimmermann, M. Ochoa-Hueso, M. Schutz, B. Frey, J.L. Firn, P.A. Fay, F. Hagedorn, E. T. Borer, E. W. Seabloom, et al. 2019. Soil net nitrogen mineralisation across global grasslands. *Nature Communications* 10:4981. doi:10.1038/s41467-019-12948-2.

- Salmeron, M., R. Isla, and J. Cavero. 2011. Effect of winter cover crop species and planting methods on maize yield and N availability under irrigated Mediterranean conditions. *Field Crops Research* 123:89–99. doi:10.1016/j.fcr.2011.05.006.
- Sierra, J. 1997. Temperature and soil moisture dependence of N mineralization in intact soil cores. *Soil Biology and Biochemistry* 29:1557–63. doi:0038-0717/97.
- Smith, K. A., T. Ball, F. Conen, K. E. Dobbie, J. Massheder, and A. Rey. 2003. Exchange of greenhouse gases between soil and atmosphere: Interactions of soil physical factors and biological processes. *European Journal of Soil Science* 54:779–91. doi:10.1046/j.1351-0754.2003.0567.x.
- Stadler, C., S. Tucher, U. Schmidhalter, R. Gutser, and H. Heuwickel. 2006. Nitrogen release from plant-derived and industrially processed organic fertilizers used in organic horticulture. *Journal of Plant Nutrition and Soil Science* 169:549–56. doi:10.1002/jpln.200520579.
- Stres, B., T. Danevcic, L. Pal, M. M. Fuka, L. Resman, S. Leskovec, J. Hacin, D. Stopar, I. Mahne, and I. Mandic-Mulec. 2008. Influence of temperature and soil water content on bacterial, archaeal and denitrifying microbial communities in drained fen grassland soil microcosm. *FEMS Microbiology Ecology* 66:110–22. doi:10.1111/j.1574-6941.2008.00555.
- Suárez, M. H., E. P. Méndez, B. R. Galdon, E. R. Rodríguez, and C. D. Romero. 2011. Influence of agronomic variables on quality of tomato fruits. *Agricultural Sciences* 2:424–31. doi:10.4236/as.2011.24054.
- Temminghoff, E. J. M., and V. J. Houba. 2004. *Plant analysis procedures second edition*. Dordrecht: Kluwer Academic Publishers.
- Wallenius, K., H. Rita, S. Simpanen, A. Mikkonen, and R. M. Niemi. 2010. Sample storage for enzyme activity and bacterial community profiles. *Journal of Microbiological Methods* 81:48–55. doi:10.1016/j.mimet.2010.01.021.
- Wallenstein, M., S. D. Allison, J. Ernakovich, J. M. Steinweg, and R. Sinsabaugh. 2011. Controls on the temperature sensitivity of soil enzymes: A key driver of *in situ* enzyme activity rates. In *Soil enzymology*. *Soil biology*, ed. G. Shukla and A. Varma, vol 22. 245–58. Heidelberg, Berlin: Springer. doi:doi.10.1007/978-3-642-14225-3\_13.
- Wang, C., S. Wan, X. Xing, L. Zhang, and X. Han. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biology and Biochemistry* 38:1101–10. doi:10.1016/j.soilbio.2005.09.009.
- Wienhold, B. J. 2007. Comparison of laboratory methods and an in-situ method for estimating nitrogen mineralization in an irrigated silt-loam soil. *Communications in Soil Science and Plant Analysis* 38:1721–32. doi:10.1080/00103620701435498.
- Wu, L., and L. Q. Ma. 2001. Effects of sample storage on biosolids compost stability and maturity evaluation. *Journal of Environmental Quality* 30:222–28. doi:10.2134/jeq2001.301222x.
- Xiang, S., A. Doyle, A. Holden, P. A. Schimel, and J. P. Schimel. 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biology & Biochemistry* 40:2281–89. doi:10.1016/j.soilbio.2008.05.004.
- Yamasaky, A., R. Taterno, and H. Shibata. 2011. Effects of carbon and nitrogen amendment on soil carbon and nitrogen mineralization in volcanic immature soil in Southern Kyushu, Japan. *Journal Forest Research* 16:414–23. doi:10.1007/s10310-011-0272-7.
- Yan, D. Z., D. J. Wang, R. J. Sun, and J. H. Lin. 2006. N mineralization as affected by long-term N fertilization and its relationship with crop N uptake. *Pedosphere* 16:125–30. doi:10.1016/S1002-0160(06)60034-9.
- Zhou, X., C. Chen, S. Lu, Y. Rui, H. Wu, and Z. Xu. 2012. The short-term cover crops increase soil labile organic carbon in southeastern Australia. *Biology and Fertility of Soils* 48:239–44. doi:10.1007/s00374-011-0594-9.
- Zinati, G. M., D. R. Christenson, and D. Harris. 2007. Estimation of field N mineralization from laboratory incubation for sugar beet production in Michigan. *Communications in Soil Science and Plant Analysis* 38:827–42. doi:10.1080/00103620701220874.
- Zucconi, F., and M. de Bertoldi. 1987. Composts specifications for the production and characterization of composts from municipal solid waste. In *Compost: Quality and use*, In, ed. M. de Bertoldi, M. P. Ferranti, P. L'Hermite, and F. Zucconi, 30–50. London: Elsevier Applied Science.