



Controlled experiment to determine nitrogen availability for seven organic fertilisers in three contrasting soils

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ABSTRACT

Organic production systems have generated new technology and management tools, including the use of different nutrient sources. To support to the selection of appropriate organic N fertilisers, based on their N availability over time, a controlled experiment was carried out in three soils with contrasting physical-chemical properties. Seven organic fertilisers, a control without fertiliser and a reference with a conventional fertiliser were used, all providing a total N dose of 100 mg kg⁻¹ soil. Soils were incubated under aerobic conditions for 7, 14, 28, 56 and 112 days, to determine the availability of ammonium, nitrate, and available N. This enabled classification of the fertilisers, according to N availability rates for the total incubation period, as fertilisers with low (compost), medium (Fertil, lupin meal and blood meal), or high (sodium nitrate, Purely Grow and Purely Lysine) N availability rates. According to the speed of N delivery, fertilisers were classified as having a rapid (Purely Grow and sodium nitrate), intermediate (Purely Lysine, blood meal and Fertil), or slow (lupin meal and compost) N delivery speed. The results can be used to support decision making in organic production by enabling the selection of N fertilisers according to their N availability rate and to adjust fertiliser applications to the requirements of the crop.

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Introduction

Organic production systems have had a sustained growth worldwide due to the nutritional quality and safety of the food produced and the positive effects on the environment (Wang et al. 2008; Karanatsidis and Berova 2009; Epule et al. 2015). The use of inputs is a key difference between organic and conventional agriculture systems. The products and techniques commonly used to supply nutrients in organic systems include compost, green manuring, legume seed meal and blood meal, and there is often special focus on the input and management of N, as this is the main nutrient determining plant productivity (Miller et al. 2006; Lin et al. 2007; Mondini et al. 2008; Nett et al. 2010; Li et al. 2015). For use in organic systems, all inputs must be approved for use in accordance with organic farming standards e.g. the National Organic Program (NOP) (USDA 2017), and from a practical point of view it is also important that the nutrient input generate

economically viable yields and improve soil quality and fertility (Chang et al. 2007; Verma et al. 2015).

The selection of appropriate organic N-based fertilisers depend on the total N content, the actual contribution of available N and the N delivery dynamics (Li et al. 2009, 2015; Muñoz-Vega et al. 2016). Regarding this, there is limited information in the literature relating to manures and compost from animal and plant origin (Hartz et al. 2000; Preusch et al. 2002; Rees and Castle 2002; Sullivan et al. 2003; Nett et al. 2010; Hirzel et al. 2012), legume grain meal (Li et al. 2009, 2015) and blood, meat and bone meal (Ciavatta et al. 1997; Jeng et al. 2006; Mondini et al. 2008).

Once an organic fertiliser has been applied to the soil, the availability of nutrients depends on the activity of the soil microflora, as the microbial biomass decomposes organic matter either to obtain nutrients or to deliver chemically bound energy to the microorganisms (Nett et al. 2010). Nitrogen mineralisation and delivery involves chemical and biological transformations that generate ammonium ($\text{NH}_4^+\text{-N}$) and nitrate (NO_3^-N), which may be more or less favorable for different cultivated species; blueberries, for example, require a higher availability of ammoniacal N and soil acidity (Retamales and Hancock 2012; Strik 2014), so the choice of an appropriate N source will also depend on the species to be cultivated.

Considering the increase in organic production worldwide and the lack of knowledge on the contribution of available N from different nutrient sources, the objective of this study was to determine the dynamics and availability of N over time from different organic fertilisers in three soils of contrasting edaphic properties and to classify these fertilisers according to the dynamic (speed) and availability rate of N.

Materials and methods

Soils

Soil samples (0–20 cm depth) were collected from three locations in south-central and south of Chile: a loam textured Fluventic Xerochrepts (35°05'45.25" S, 72°01'21.28" W); a sandy loam textured Aquic Haploxerolls (37°27'35.81" S, 72°30'02.04" W) and a silty loam textured Melanudands (39°11'53" S, 72°15'38" W) (CIREN 1997, 1999, 2002; USDA 2014). The physical-chemical characteristics of the soils have been listed in Table 1. Chemical properties were analysed

Table 1. Physical and chemical properties of the soils (collected at 0–20 cm depth) prior to the experiment.

Parameter	Xerochrepts	Haploxerolls	Melanudands
Clay, %	10.8	8.3	23.0
Silt, %	48.4	43.2	57.6
Sand, %	40.8	48.5	19.4
Water retention to 33 kPa, %	19.10	19.15	83.89
Water retention to 1500 kPa, %	10.17	12.09	56.45
Bulk density, g cm^{-3}	1.33	1.31	0.56
pH (soil: water 1: 5)	5.94	6.55	5.72
Organic matter, g kg^{-1}	44.5	42.2	282.7
Electrical conductivity, dS m^{-1}	0.11	0.07	0.13
Available N, mg kg^{-1}	36.5	23.4	76.0
Available P, mg kg^{-1}	48.0	14.0	11.8
Exchangeable K, $\text{cmol}_+ \text{kg}^{-1}$	0.61	0.42	0.36
Exchangeable Ca, $\text{cmol}_+ \text{kg}^{-1}$	7.16	5.97	10.08
Exchangeable Mg, $\text{cmol}_+ \text{kg}^{-1}$	2.29	1.58	0.85
Exchangeable Na, $\text{cmol}_+ \text{kg}^{-1}$	0.61	0.34	0.08
Exchangeable Al, $\text{cmol}_+ \text{kg}^{-1}$	0.03	0.03	0.09
Available Fe, mg kg^{-1}	206.00	50.27	69.15
Available Mn, mg kg^{-1}	10.87	2.82	3.89
Available Zn, mg kg^{-1}	2.70	0.71	3.78
Available Cu, mg kg^{-1}	6.01	1.48	1.08
Available B, mg kg^{-1}	0.22	0.30	0.38
Available S, mg kg^{-1}	15.9	3.0	16.1

by the methods described in Sadzawka et al. (2006). Soil pH was measured in a 1: 2.5 soil: water solution ratio with a pH electrode. Soil organic matter (OM) was measured by the Walkley-Black wet digestion method (Walkley and Black 1934). Electrical conductivity was measured using a conductivity cell (soil: water ratio 1:5). Soil available N (NO_3^- -N and NH_4^+ -N) was extracted with 2 mol L^{-1} KCl and determined by colorimetry in a Skalar autoanalyser (segmented flux spectrophotometer) (Skalar Ltd, York, UK). Available P in the soil sample was extracted using 0.5 M NaHCO_3 and determined using ascorbic acid-molybdate (Olsen et al. 1954). Exchangeable Ca, Mg, K, and Na were determined using 1 M ammonium acetate extraction followed by flame spectroscopy, that is absorption (Ca and Mg) and emission (K and Na). Soil exchangeable Al concentration was determined by 1 M KCl extraction with absorption spectroscopy. Soil Fe, Mn, Zn, and Cu concentrations were determined in diethylenetriaminepentaacetic acid (DTPA) extract by atomic absorption spectrometry (AAS) (Lindsay and Norvell 1978). Boron was determined by colorimetry in a solution obtained after hot water extraction. Available S in the soil was extracted with calcium phosphate and determined by turbidimetry. Soil texture was analysed by the Bouyoucos hydrometer method (Bouyoucos 1962). Bulk density was determined by the cylinder method on five cores per soil.

The properties of the soils (Table 1) differed in terms of source materials, formation factors, and humidity-temperature regimes, as observed in their taxonomic classifications (Xerochrepts, Haploxerolls and Melanudands; data not shown), as well as with regard to clay content, bulk density, moisture retention capacity, organic matter content, and some chemical properties such as pH and initial concentrations of N, P, Fe, Mn, Zn and S. Some properties, such as texture, organic matter content and Fe are known to affect the natural mineralisation capacity of N associated to a higher or lower activity of the microbial biomass through chemical and biological processes that involve oxidation-reduction reactions and C and N mineralisation (Sahrawat and Narteh 2001; Bushong et al. 2007; Hirzel and Stolpe 2015).

Fertiliser treatments

Nine fertiliser treatments were evaluated, chosen to reflect the organic fertilisers available on the local market: 1) A control without fertiliser (CT) as an indicator of the nutrient supply from the soil; 2) Compost (CO), compost produced outdoors in static windrows/piles using shredded agricultural plant residues as feedstock; 3) Fertil (F) protein N pellets from enzymatic hydrolysis (ILSA S.P.A., Arzignano, Italy); 4) Purely Grow (PG) plant-based proteins and hydrolysed fish protein concentrate liquid (Purely Organics Products, Portsmouth, New Hampshire, USA); 5) Purely Lysine (PL) plant-based proteins and hydrolysed fish protein concentrate mini-pellets (Vita Flex, Phoenix, Arizona, USA); 6) blood meal (BM) powder, 7) Lupin meal (LM), ground grain; 8) Sodium nitrate (SN) K-enriched granules (N: P_2O_5 : K_2O ; 15.0: 0.0: 9.0); and 9) Conventional fertiliser granules (CF) with urea, triple superphosphate and potassium sulfate. The nutrient characteristics of the fertilisers have been described in Table 2. The fertilisers used for treatments 2, 3, 4, 5, 6, 7 and 8 were all approved for use in organic agriculture in accordance with the National Organic Program (NOP) (USDA 2017). Although sodium nitrate (treatment 8) is approved for use by NOP, it was recognized that this input is different in physical and chemical nature in relation to both its origin and extraction process and is not permitted for use by some other organic farming standards. The rate of N applied to all the treatments, except CT, was equivalent to 100 mg N kg^{-1} . All solid fertilisers (granules and pellets) were ground using a pestle and mortar (<60 mesh) and all liquid fertilisers were diluted to 10% v/v in distilled water, which, likely, increased their real N availability to be tested under controlled conditions over a limited period of time. The fertilisers were individually mixed with the soil samples and placed into 0.25 L plastic jars for incubation, using 24 replicates per treatment. The initial total N concentration of the fertilisers varied according to the source, as well as their initial NH_4^+ -N and NO_3^- N concentration, which is described in Table 2.

Table 2. Characteristics and nutrient composition of the fertilisers used in this study.

Fertiliser	Nutrient concentration (%)					Relation C: N	Formulation
	N	P ₂ O ₅	K ₂ O	NH ₄ ⁺ -N	NO ₃ ⁻ N		
Compost	0.84	0.80	0.45	5.5*10 ⁻⁵	1.9*10 ⁻³	11.96	Powder
Fertil	12.00	0.00	0.00	0.16	0.00	7.60	Pellet
Purely Grow	13.10	0.00	4.00	1.42	0.00	6.20	Liquid
Purely Lysine	15.50	0.00	0.00	0.77	0.00	5.80	Pellet
Blood meal	14.25	0.70	0.60	0.01	0.00	3.74	Powder
Lupine meal	7.93	0.90	1.00	0.03	0.00	5.67	Ground grain
Sodium nitrate	15.00	0.00	9.00	0.00	15.00	-	Granules
Mixture of urea, triple superphosphate and potassium sulfate	23.23	8.15	15.33	0.00	0.00	-	Granules

Soil incubation

Samples of individually homogenised soils (100 g) were placed into 0.25 L plastic jars, moistened to 80% of their water holding capacity and incubated aerobically at 25 ± 2°C, in a refrigerated incubator (FOC 225E, Velp Scientifica, Usmate, Italy) for 16 weeks. The jars were left opened for 1 h and soil moisture adjusted gravimetrically every week (Hirzel et al. 2010). At each sampling date (after 0, 7, 14, 28, 56 and 112 days (d)), four replicates of each fertiliser treatment were randomly selected for analysis of NH₄⁺-N, NO₃⁻N and inorganic N (sum of NH₄⁺-N and NO₃⁻N) was calculated. NH₄⁺-N and NO₃⁻N, analyses were carried out in a Skalar autoanalyser (segmented flux spectrophotometer) (Skalar Ltd, York, UK) on 5 g of soil sample.

Determination of rate and dynamics of n availability

For each soil and fertiliser treatment, N availability rate was calculated as the average concentration of NH₄⁺-N + NO₃⁻N throughout the incubation time, to allow calculation of inorganic N for the total incubation period minus the concentration of inorganic N of the control without fertiliser, divided by the amount of total N applied at the start of the experiment (100 mg kg⁻¹), and then multiplied by 100 to express the result as a percentage (Equation 1):

$$\text{Rate of N availability (\%)} = [(N_f - N_c) / 100] * 100 \quad (1)$$

where, N_f is average concentration of inorganic N of a fertiliser treatment, and N_c is average concentration of inorganic N of the control treatment.

Using the rate of N availability, obtained as an average for the total incubation period, treatments were arbitrarily rated as fertilisers with low (lower than 30%), medium (between 31 and 60%) or high (higher than 61%) N availability, aiming to facilitate the selection of a N-based fertiliser and its dose of application for certain stages of crop development, based on this parameter.

The speed of N delivery was determined as the sum of NH₄⁺-N + NO₃⁻N at each evaluation time (0, 7, 14, 28, 56 and 112 d) and adjusted to a second order polynomial model. The fertiliser treatments were arbitrarily classified as having a slow, intermediate or rapid N delivery speed according to the percentage of N available after the first 7 and 28 days of incubation. So, treatments with a N availability rate higher than 60% at 7 incubation days were classified as rapidly available fertilisers; fertiliser treatments with a N availability rate higher than 60% at 28 days of incubation were classified as medium, and treatments with a N availability rate lower than 60% after 28 days of incubation were classified as slowly available fertilisers.

Statistical analysis

Considering the differences in the physical-chemical properties of the three soils used in this experiment, the experimental design was a split-plot design for each soil, where the incubation time was the main plot and the fertiliser treatment was the sub-plot. One- and two-way ANOVAs, mean separation test (Tukey), and separation of interactions by contrasts were performed at the 5% significance level, using SAS 8.0 (SAS Institute 1999). Contrast analysis was used to separate the interactions obtained.

Results

Xerochrepts soil

In the Xerochrepts soil, incubation time and fertiliser treatment had significant effects ($p < 0.01$; Table 3). In turn, the interactions were also significant ($p < 0.01$; Table 3). Therefore, to separate the effect of fertiliser treatment, a contrast analysis was performed (Table 4). In addition, the incubation time effect and the interaction with fertiliser treatment are shown in Figures 1(a-c), for the evolution of $\text{NH}_4^+\text{-N}$, NO_3^-N , and available N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{N}$) concentrations, respectively.

The contrast between fertiliser treatments indicated that for $\text{NH}_4^+\text{-N}$ concentration, the highest values were obtained with PG and PL treatments ($p < 0.05$), and lower values were obtained with

Table 3. Results of two-way ANOVA for available N, $\text{NH}_4^+\text{-N}$ and NO_3^-N concentration, considering incubation time (T), treatment (F) and their interaction (T \times F) for each soil type.

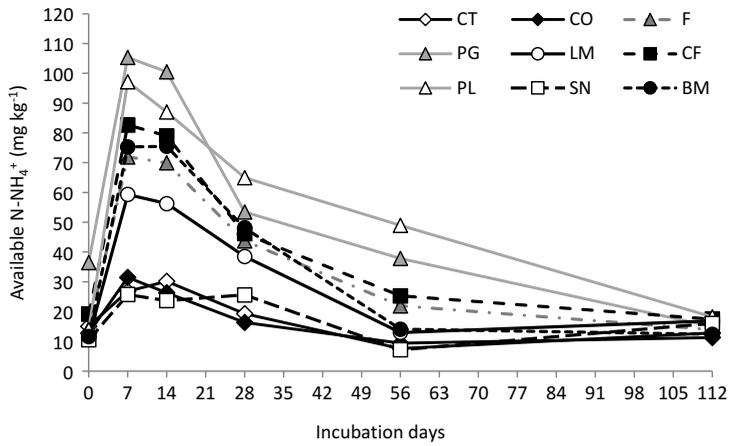
Parameter	Incubation time (T)	Treatment (F)	Interaction T \times F
Xerochrepts soil			
Available N	**	**	**
$\text{NH}_4^+\text{-N}$	**	**	**
NO_3^-N	**	**	**
Haploxerolls soil			
Available N	**	**	**
$\text{NH}_4^+\text{-N}$	**	**	**
NO_3^-N	**	**	**
Melanudands soil			
Available N	**	**	**
$\text{NH}_4^+\text{-N}$	**	*	**
NO_3^-N	**	**	**

Significances are: * $p < 0.05$; ** $p < 0.01$

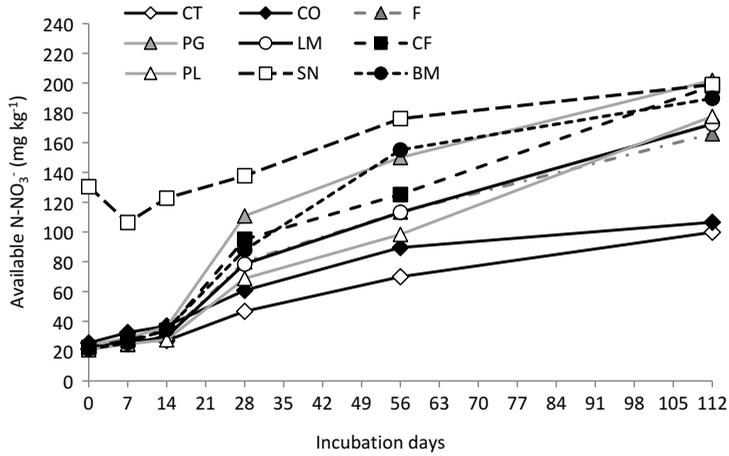
Table 4. Contrast analysis for distinction of available N (average values for the incubation period) between the fertiliser treatments in each soil.

Parameter	CT	CO	F	PG	PL	BM	LM	SN	CF
Xerochrepts soil									
$\text{NH}_4^+\text{-N}$, mg kg^{-1}	18.6 e	17.9 e	39.3 c	58.1 a	55.0 a	39.5 c	32.5 d	18.1 e	44.9 b
NO_3^-N , mg kg^{-1}	48.7 e	58.6 d	73.0 c	92.0 b	69.7 c	85.7 b	73.6 c	145.4 a	83.6 b
Available N, mg kg^{-1}	67.3 e	76.6 e	112.2 d	150.0 b	124.7 c	125.2 c	106.1 d	163.5 a	128.6 c
Haploxerolls soil									
$\text{NH}_4^+\text{-N}$, mg kg^{-1}	13.9 d	13.2 d	28.3 c	57.6 a	43.6 b	38.8 b	29.2 c	15.7 d	44.3 b
NO_3^-N , mg kg^{-1}	39.4 e	41.2 e	64.9 d	83.6 b	70.9 c	71.7 c	61.0 d	122.2 a	73.6 c
Available N, mg kg^{-1}	53.3 d	54.4 d	93.3 c	141.2 a	114.4 b	110.5 b	90.2 c	137.9 a	117.9 b
Melanudands soil									
$\text{NH}_4^+\text{-N}$, mg kg^{-1}	27.3 ab	29.1 ab	33.5 ab	37.3 ab	38.5 a	36.0 ab	23.7 b	24.8 ab	36.6 ab
NO_3^-N , mg kg^{-1}	129.0 e	143.9 e	172.9 cd	209.2 ab	183.8 cd	175.9 cd	171.8 d	212.3 a	191.0 bc
Available N, mg kg^{-1}	156.3 f	173.1 f	206.4 de	246.5 a	222.3 bcd	211.9 cde	195.5 e	237.1 ab	227.6 bc

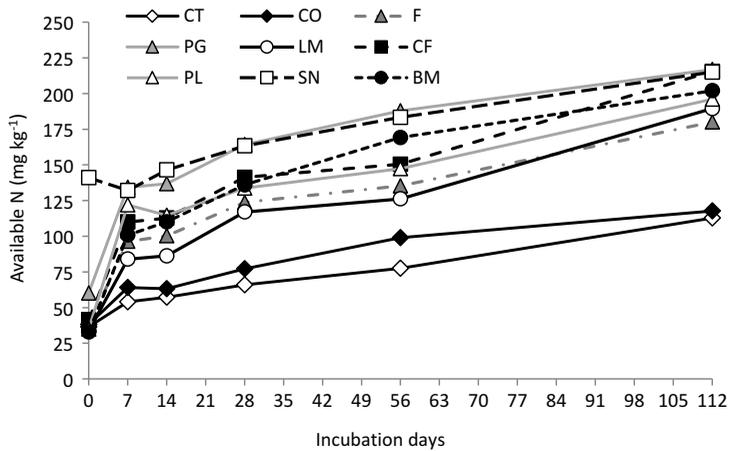
Different letters in the same row indicate significant differences between treatments according to Tukey's test ($p < 0.05$). Abbreviations represent: CT, control without fertiliser; CO, compost; F, Fertil; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser.



a



b



c

Figure 1. Evolution of NH₄⁺-N, NO₃⁻-N, and available N concentrations during the incubation time in Xerochrepts soil. CT, control without fertiliser; CO, compost; F, Fertil; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser.

CT, CO and SN treatments ($p < 0.05$) (Table 4). The conventional fertiliser had a lower NH_4^+ -N concentration than PG and PL treatments ($p < 0.05$), but higher than the other treatments ($p < 0.05$) (Table 4). For NO_3^- -N concentration, the highest value was obtained with SN ($p < 0.05$) (Table 4). The lowest concentration was obtained in the CT treatment ($p < 0.05$) (Table 4). The available N concentration was highest in the SN treatment ($p < 0.05$) (Table 4). The lower values were found in CT and CO treatments ($p < 0.05$) (Table 4).

In general, the variations in NH_4^+ -N concentration were associated with the nitrification process that generated an increase in the concentration of NO_3^- -N (Figure 1(a,b)). The evolution of NO_3^- -N, tended to increase in all fertiliser treatments (Figure 1(b)), with a marked difference at the start of the experiment in the SN treatment and an increase from 14 to 112 days in F, PG, LM, CF, PL and BM treatments. These differences of magnitude in the increase of NO_3^- -N concentration between treatments across the incubation time were detected with the interaction analysis (Table 3). For the evolution of the available N concentration (Figure 1(c)), a similar effect as that described for the NO_3^- -N concentration was observed (Table 3).

The rate of N availability (Figure 4(a)) of each fertiliser ranged between 9.2% and 96.2% (average 56.0%). The highest value was obtained with the use of SN ($p < 0.05$) (Figure 4(a)). The lowest real N availability was obtained with the use of CO ($p < 0.05$) (Figure 4(a)).

The quadratic regression model used to estimate the availability of N showed an adequate adjustment in Xerochrepts for most of the treatments, with coefficients of determination higher than 80% (Table 5). In the case of the non-fertilised control (baseline net mineralisation indicator for each soil), the determination coefficient was 96.7% (Table 5), indicating high validity of the model for this soil.

Regarding N delivery speed (Table 6; Figure 1(c)), the organic fertilisers PG, PL and SN showed a rapid N delivery speed (more than 60% of net N availability at 7 d of soil incubation),

Table 5. Components of the polynomial equation ($Y = a + b*X - c*X^2$) adjusted to estimate the evolution of available N of the fertiliser treatments with respect to the incubation time (d) in the three soils.

Soil	Treatment	Determination coefficient (R^2)	a	b	c	
Xerochrepts	CT	0.967	43.70	0.752	0.0013	
	CO	0.963	45.68	1.325	0.0061	
	F	0.873	63.07	2.115	0.0098	
	PG	0.874	89.38	2.922	0.0162	
	PL	0.774	73.08	2.233	0.0104	
	LM	0.915	55.18	1.928	0.0067	
	BM	0.920	58.68	3.121	0.0166	
	SN	0.971	133.95	1.020	0.0026	
	CF	0.870	72.01	2.263	0.0091	
	Haploxyerolls	CT	0.902	30.94	1.119	0.0065
		CO	0.887	29.15	1.334	0.0083
F		0.896	43.11	2.791	0.0182	
PG		0.775	104.98	1.808	0.0107	
PL		0.653	68.98	2.366	0.0144	
LM		0.704	52.63	2.087	0.0136	
BM		0.692	61.45	2.565	0.0157	
SN		0.395	118.20	1.170	0.0081	
CF		0.661	83.75	1.839	0.0116	
Melanudands		CT	0.729	105.00	2.869	0.0188
		CO	0.656	117.83	3.053	0.0198
	F	0.838	118.98	4.898	0.0322	
	PG	0.889	131.86	6.406	0.0420	
	PL	0.826	132.49	5.250	0.0359	
	LM	0.969	94.75	5.407	0.0327	
	BM	0.819	123.11	4.705	0.0292	
	SN	0.934	169.94	3.815	0.0254	
	CF	0.968	148.18	4.850	0.0345	

CT, control without fertiliser; CO, compost; F, Fertile; PG, Purely Grow; PL, Purely Lysine; LM: lupine meal; BM: blood meal; SN: sodium nitrate; CF: conventional fertiliser.

In the equation Y is available N (mg kg^{-1}) and X is the incubation time (d).

Table 6. Percentage of N availability ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{N}$) after 7 and 28 days of soil incubation for each fertiliser treatment in the three soils.

Soil	Treatment	Incubation time	
		7 d	28 d
%			
Xerochrepts	CO	10	11
	F	42	58
	PG	80	98
	PL	68	68
	LM	30	51
	BM	47	70
	SN	78	97
	CF	56	75
	CO	2	2
Haploxeerolls	F	25	48
	PG	80	84
	PL	61	67
	LM	41	44
	BM	48	68
	SN	79	84
	CF	68	68
	CO	21	30
	F	30	82
Melanudands	PG	21	100
	PL	32	100
	LM	0	68
	BM	33	81
	SN	41	98
	CF	28	97

CT, control; CO, compost; F, Fertile; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser.

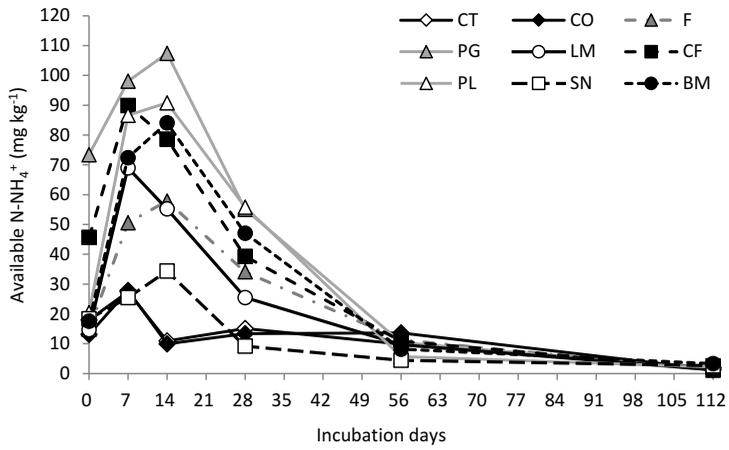
while the BM fertiliser had intermediate N delivery speed (greater than 60% of net N availability at 28 d of soil incubation), and CO, F and LM fertilisers showed a slow N delivery speed (less than 60% of N net availability at 28 d of soil incubation). It is important to underline the slow N delivery speed found in the CO treatment (10% at 7 days and 11% at 28 days of incubation; Table 6).

Haploxeerolls soil

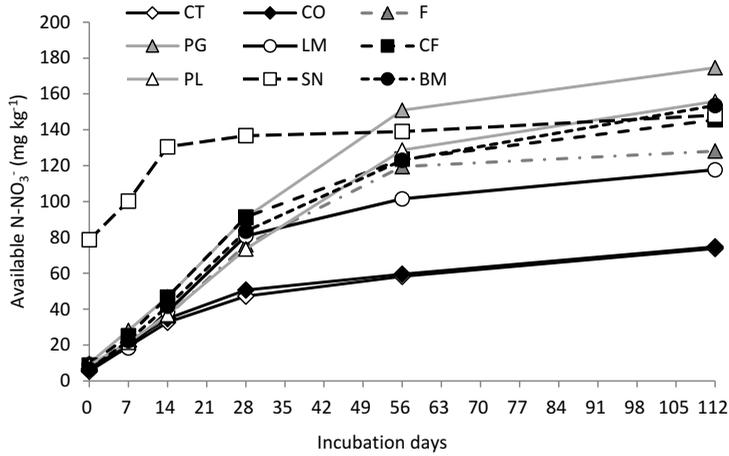
In the Haploxeerolls soil, incubation time and fertiliser treatment had significant effects ($p < 0.01$; Table 3), and the interactions were also significant ($p < 0.01$; Table 3). Therefore, to separate the effect of fertiliser treatment, contrast analysis was performed (Table 4). In addition, the incubation time effect and the interaction with fertiliser treatment are described in Figure 2(a–c), for evolution of $\text{NH}_4^+\text{-N}$, NO_3^-N , and available N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{N}$) concentrations, respectively.

The contrast between the fertiliser treatments indicated that for $\text{NH}_4^+\text{-N}$ the highest value was obtained with PG ($p < 0.05$) (Table 4). The lowest values were found in CT, CO and SN ($p < 0.05$). For NO_3^-N , the highest concentration was obtained in the SN treatment ($p < 0.05$) (Table 4), and the lowest values in the CT and CO treatments ($p < 0.05$) (Table 4). For the available N concentration, the higher values were found in SN and PG treatments ($p < 0.05$) and lower concentrations were found in CT and CO treatments ($p < 0.05$) (Table 4).

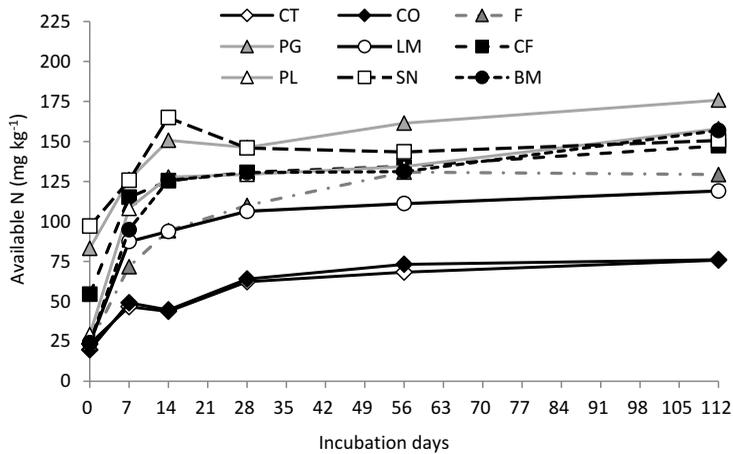
For the evolution of the $\text{NH}_4^+\text{-N}$ concentration (Figure 2(a)), treatments CT, CO, LM and CF had the higher values at 7 days of incubation, while treatments PG, PL, BM, F and SN showed their higher values at 14 days of incubation. Thereafter, all treatments showed a decrease over the time, with differences of magnitude between times associated to the interaction between incubation time \times fertiliser treatments (T \times F) (Table 3). The evolution of NO_3^-N concentration tended,



a



b



c

Figure 2. Evolution of NH₄⁺-N, NO₃⁻-N, and available N concentrations during the incubation time in Haploxerolls soil. CT, control without fertiliser; CO, compost; F, Fertil; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser.

in general, to increase in all fertiliser treatments (Figure 2(b)), with a marked difference at the start of the experiment in the SN treatment and an increase from 0 to 28 days in F, PG, LM, CF, PL and BM. These differences of magnitude in the evolution of $\text{NO}_3^- \text{N}$ concentration among treatments over the incubation time were detected through the interaction analysis (Table 3). For the evolution of the available N (Figure 2(c)), the majority of the treatments had a continuous increase during the incubation time, except in the SN treatment with its highest concentration at 14-days. Differences of magnitude in the increase of available N concentration between treatments across the incubation time (Figure 2(c)) were detected with the interaction analysis (Table 3).

The rate of N availability (Figure 4(b)) of each fertiliser ranged between 1.1% and 87.3% (average 54.1%). The highest values were obtained with the use of SN and PG ($p < 0.05$) (Figure 4(b)). The lowest real N availability value was obtained with the use of CO ($p < 0.05$) (Figure 4(b)).

The quadratic regression model used to estimate the availability of N showed that for most of the treatments the determination coefficients were less than 80%, indicating moderate validity of the model for this soil (Table 5). In the case of the non-fertilised control (baseline net mineralisation indicator for each soil), the determination coefficient was 90.2% (Table 5).

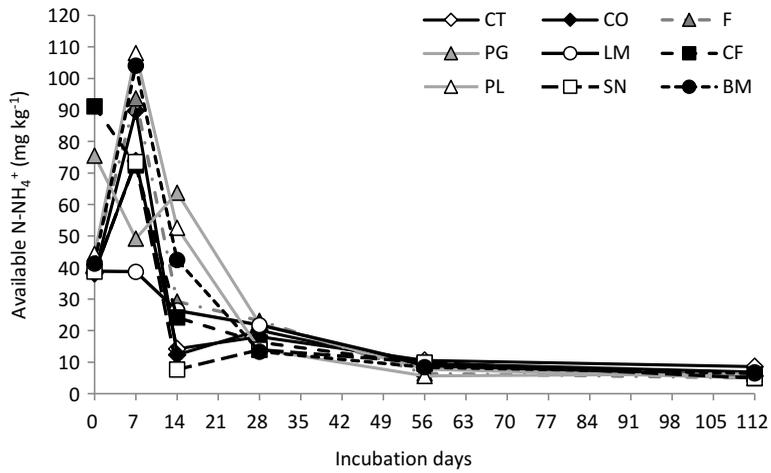
Regarding N delivery speed (Table 6; Figure 2(c)), the organic fertilisers PG, PL and SN showed a rapid N delivery speed (more than 60% of net N availability at 7 d of soil incubation), while the BM fertiliser showed an intermediate N delivery speed (greater than 60% of net N availability at 28 d of soil incubation), and CO, F and LM fertilisers had a slow N delivery speed (less than 60% of N net availability at 28 d of soil incubation), where CO stands out reporting a 2% of N availability at 7 and 28 days of incubation (Table 6).

Melanudands soil

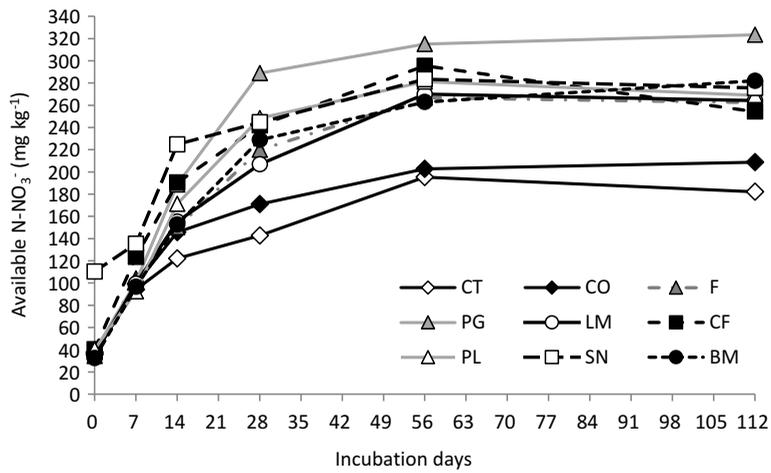
In the Melanudands soil, incubation time and fertiliser treatment had significant effects ($p < 0.05$ and $p < 0.01$; Table 3). Interactions were also significant ($p < 0.01$, Table 3). Therefore, to separate the effect of the fertiliser treatment, a contrast analysis was performed (Table 4). In addition, the incubation time effect and the interaction with fertiliser treatment are described in Figures 3(a–c), for the evolution of $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{N}$, and available N ($\text{NH}_4^+ \text{-N} + \text{NO}_3^- \text{N}$) concentrations, respectively.

The differences between the fertiliser treatments indicated that the highest $\text{NH}_4^+ \text{-N}$ concentration was obtained in PL treatment ($p < 0.05$) (Table 4), though this was only significantly different to that in the LM treatment. For the $\text{NO}_3^- \text{N}$ concentration, the highest concentration was obtained with SN ($p < 0.05$), and the lowest values were obtained in CT and CO treatments ($p < 0.05$) (Table 4). The available N concentration was higher in PG ($p > 0.05$). The lower values were obtained in CT and CO treatments ($p < 0.05$) (Table 4).

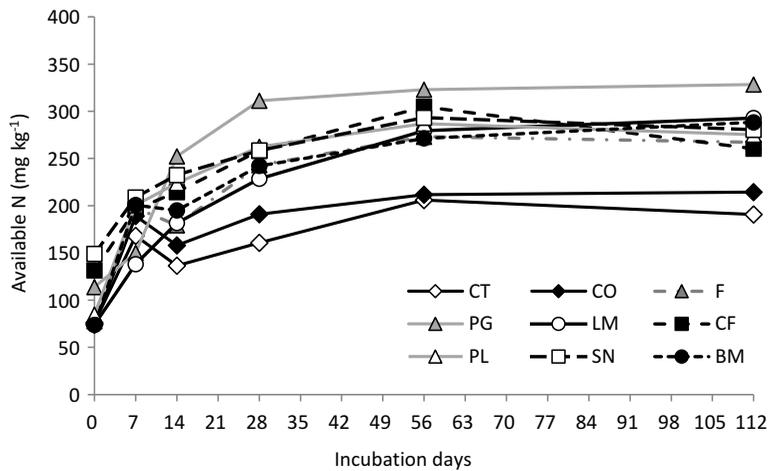
The evolution of $\text{NH}_4^+ \text{-N}$ concentration (Figure 3(a)) showed the highest values in the CF and PG treatments at the start of the incubation time. In general, $\text{NH}_4^+ \text{-N}$ concentrations decreased across the incubation time in all treatments (Figure 3(a)), except in the PG treatment at the 14-day, with differences of magnitude between times and treatments associated to their interactions (Table 3). The evolution of $\text{NO}_3^- \text{N}$ concentration tended to increase in all fertiliser treatments (Figure 3(b)), with a marked difference at the start of the experiment in the SN and a strong increase from 0 to 28 days in all the rest of the treatments. The differences of magnitude between treatments across the incubation time were detected by the interaction analysis (Table 3). For the evolution of the available N concentration (Figure 3(c)), most of the treatments showed a continuous increase during the incubation time, except in CT, CO and F treatments with a decrease at the 14-day. In general, the higher value of N concentration in all the treatments was reported at 56-days of incubation (Figure 3(c)). Differences of magnitude in the dynamic of



a



b



c

Figure 3. Evolution of NH₄⁺-N, NO₃⁻-N, and available N concentrations during the incubation time in Melanudands soil. CT, control without fertiliser; CO, compost; F, Fertili; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser.

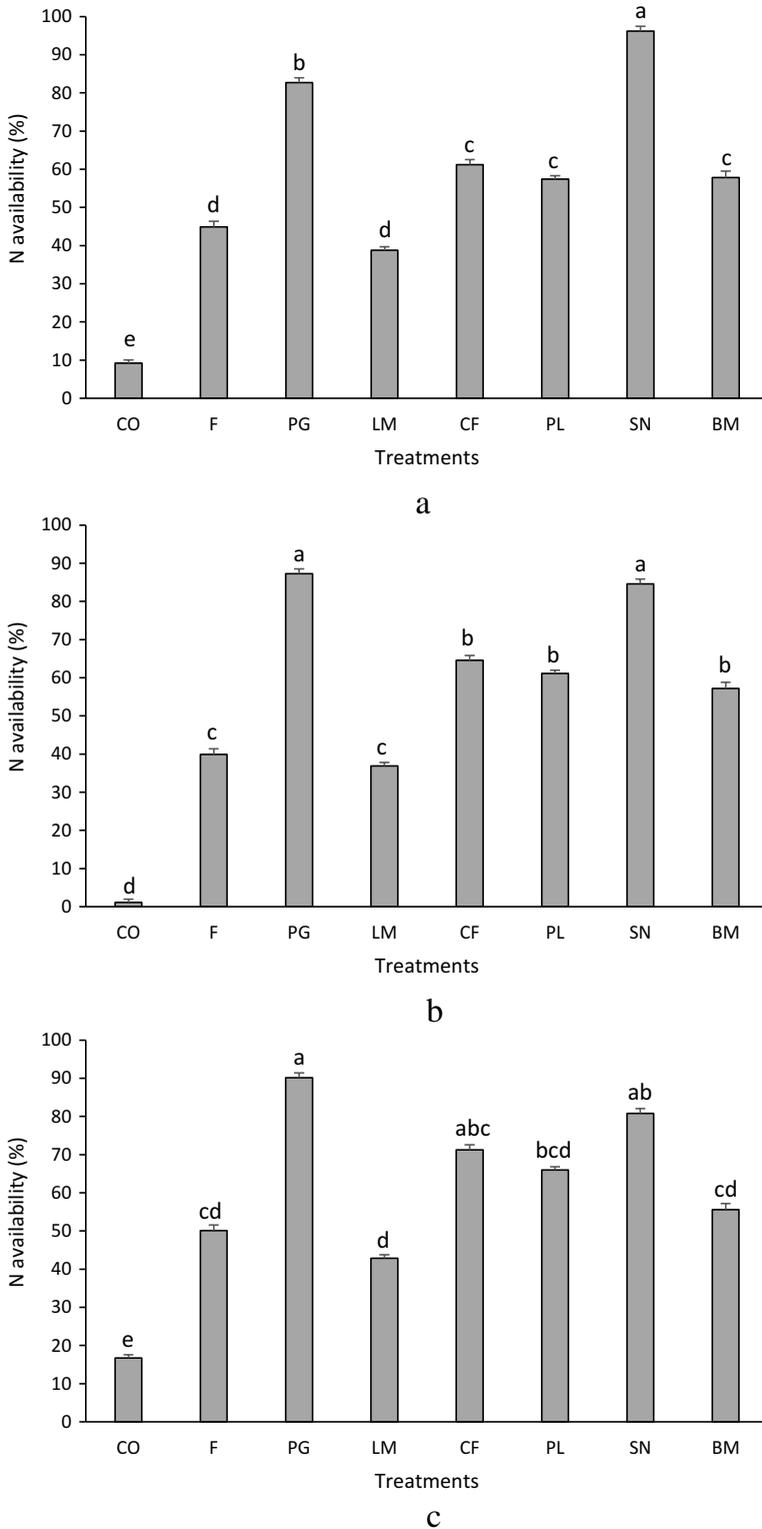


Figure 4. Rate of nitrogen availability of each fertiliser treatment as the average over the incubation period in: (a) Xerochrepts soil, (b) Haploxerolls soil, (c) Melanudands soil.

CT, control without fertiliser; CO, compost; F, Fertil; PG, Purely Grow; PL, Purely Lysine; LM, lupine meal; BM, blood meal; SN, sodium nitrate; CF, conventional fertiliser. Different letters above the bars indicate significant differences between treatments according to Tukey's test ($p < 0.05$). Error bars represent the standard error of the mean.

available N concentration between treatments across the incubation time (Figure 3(c)) were detected in the interaction analysis (Table 3).

The rate of N availability of each fertiliser ranged between 16.7% and 90.1% (average 59.2%) (Figure 4(c)). The highest values were obtained with the use of PG, SN and CF treatments ($p < 0.05$) and the lowest real N availability value was obtained with the use of CO ($p < 0.05$) (Figure 4(c)).

The quadratic regression model used to estimate N availability showed an adequate adjustment for most of the treatments, with coefficients of determination higher than 80% (Table 5). In the case of the non-fertilised control (baseline net mineralisation indicator for each soil), the determination coefficient was 72.9%, indicating moderate validity of the mathematical model (Table 5).

Regarding N delivery speed (Table 6; Figure 3(c)), in Melanudands soil (high OM content; Table 1) the trend was different that in the other soils; none of the evaluated fertilisers had a rapid N delivery speed according to the classification criteria, while fertilisers F, PG, PL, LM, BM and SN reported to have an intermediate N delivery speed (Table 6, Figure 3(a,b)).

Rates and dynamics of N availability for organic fertilisers based on the average of the three soils

According to these results and the average value of the real N availability rate obtained in the three soils, the organic fertilisers SN, PG and PL were classified with a high N availability rate (Figure 4(a-c); Table 6). Fertilisers F, LM and BM were classified with a medium N availability rate, while CO was classified with a low N availability rate (Figure 4(a-c); Table 6).

As an average of the three soils evaluated at 7 d incubation, the N availability of the organic fertilisers SN, PG, PL, F, LM, BM and CO were 66.0%, 60.3%, 53.6%, 32.3%, 23.7%, 42.7%, and 11.0%, respectively (Table 6). Similarly, at 28 d of soil incubation, and as average of the three soils, fertilisers SN, PG, PL, F, LM, BM and CO had an N availability of 93.0%, 94.0%, 78.0%, 62.7%, 54.3%, 73.0%, and 14.3%, respectively (Table 6). According to these results, PG and SN showed a rapid N delivery speed, PL, BM and F an intermediate N delivery speed, while LM and CO a slow N delivery speed.

Discussion

The physical-chemical properties of the soils in this study (Table 1) affected the rates of mineralisation, ammonification, nitrification and immobilisation of N, as described by the interactions incubation time \times fertiliser treatment (T \times F) (Tables 3 and 4, Figures 1–3). This was likely to be associated with the OM concentration favouring the biological processes in the Melanudands soil (Table 4, Figure 3(a-c)) (Preusch et al. 2002; Rasul et al. 2009; Hirzel et al. 2010; Nett et al. 2010). The higher content of sand in Xerochrepts and Haploxerolls soils (Table 1) likely also enabled a greater rate of OM decomposition due to the smaller amount of variable-charge clay minerals, providing less physical protection for the OM (Silva et al. 2016). In turn, the chemical stability of the aggregates in the soils was also affected by low or medium Fe concentrations, as in Haploxerolls and Melanudands (Table 1), which can benefit OM mineralisation (Silva et al. 2016). Hence, the soil effect could explain the higher part of the interactions previously indicated for the rates of mineralisation, ammonification, nitrification and immobilisation of N (Table 3).

The N availability rate from the different fertilisers was likely to be associated to their C: N ratio, soluble C: soluble N ratio, polyphenol concentration, and the influence of these factors on the turnover of N (Hartz et al. 2000; Seneviratne 2000; Palm et al. 2001; Bushong et al. 2007; Li et al. 2015). The nitrification rate of NH_4^+ -N (Table 4, Figures (1–3)) followed a normal-to-expected pattern (Laos et al. 2000; Chu et al. 2005; Hirzel et al. 2010) with differences between soils associated with their physical-chemical properties (Table 1), highlighting a higher rate of nitrification in the Melanudands soil (Figure 3(a-b)), likely linked to the higher biomass activity

generated in this soil (Chu et al. 2005; Nett et al. 2010; Silva et al. 2016). This effect was also observed in the greater concentration of available N in this soil (Figure 3 (c)). Regarding the base mineralisation rate of each soil measured in the control treatment, a higher mineralisation was observed in the Melanudands, associated with properties of this soil, as discussed previously (Chu et al. 2005; Nett et al. 2010; Silva et al. 2016).

The differences in the rate of N availability obtained for each organic fertiliser (Table 4) could be primarily associated to the C: N ratio (Table 2), then to polyphenol composition of each product (Bushong et al. 2007; Mohanty et al. 2013), and also to the soil characteristics (Table 1) (Preusch et al. 2002; Rasul et al. 2009; Hirzel et al. 2010; Nett et al. 2010). Moreover, other properties of the carbon in the organic matter, such as its biochemical characteristics and its cellulose and lignin composition, could also have affected the mineralisation of organic N (Mohanty et al. 2013; Li et al. 2015), though this was not analysed in this experiment. Since the net availability of N was determined in relation to a control, none of the treatments managed to generate a rate of 100% for the period of study, associated with immobilisation and adsorption processes of N in the soil organic-mineral compounds (Hartz et al. 2000; Preusch et al. 2002; Chu et al. 2005). The lowest rate of N availability was obtained in the CO, and in plant materials such as LM. These rates were in agreement with the values indicated for these types of material by other researchers (Boechat et al. 2013; Rogers et al. 2001), but lower than that reported by Li et al. (2009, 2015) for LM. However, in PG, PL and BM, the higher rate of N availability was associated with both their low C: N ratios (Table 2) and high concentrations of protein and amino acids (Sánchez et al. 2007; Farfán and Gordón 2013). The high N availability of BM was also reported by Ciavatta et al. (1997) and Mondini et al. (2008) for meat and bone meal. However, the values of N availability for BM, indicated by Ciavatta et al. (1997), were higher than those obtained in this experiment. For SN, the high rate of N availability corresponded to its fraction of $\text{NO}_3^- \text{N}$ (100% of its N content) (Table 2).

The second-degree polynomial equations used to estimate the evolution of available N from different fertilisers showed, in general, an adequate adjustment (Table 5). Although there are more complex and user friendly models to estimate evolution of N availability, either from the mineralisation of the soil or from fertilisers or organic amendments (Eghball 2000; Benítez et al. 2003; Bushong et al. 2007; Hirzel et al. 2010), the use of simple linear and polynomial models has enabled adequate adjustment also in other experiments (Dao and Cavigelli 2003; Haney et al. 2004; Hirzel and Stolpe 2015; Li et al. 2015). A comparison of the three soils without any fertiliser (CT) showed that the Melanudands soil had the highest initial N availability concentration as well as the highest slope (b value) in the polynomial equation, while the Xerochrepts soil registered an initial N availability concentration in between Melanudands and Haploxerolls soils, and the lowest slope of the three soils in the polynomial equation (Table 5). A similar trend was found in all the fertiliser treatments, with some differences between treatments PG, BM and CF (Table 5) in Xerochrepts and Haploxerolls soils, which could be associated to their contrasting physical-chemical characteristics (Table 1).

Regarding N availability from the different organic fertilisers (Table 6), there was a coincidence between N availability in the short term (7 to 28 d of incubation) (Figures (1–3c)) and the net N availability rate (Figure 4 (a–c)), since both were affected by the same factors such as the C: N ratio and polyphenol composition of the products (Hartz et al. 2000; Seneviratne 2000; Palm et al. 2001; Bushong et al. 2007; Li et al. 2015), although the latter was not evaluated in this study. In general, a lower N availability rate was obtained in the Melanudands soil in the first 7 d, likely linked to the immobilisation processes associated with the higher microbial activity of this soil with high a OM content, as well as the adsorption of N in organic-mineral compounds of the soil (Table 1) (Hartz et al. 2000; Preusch et al. 2002; Chu et al. 2005; Mohanty et al. 2013). The general classification of the fertilisers according to their rate of N availability and N availability over a specific period of time, could enable the selection of fertilisers for organic production systems in

accordance with the cost of the N unit (price per kg of product/(% N/100 × net N availability rate/100)). They could also be classified in accordance with the growing season: for application prior to planting for fertilisers with a slow N delivery speed (CO, F and LM); application prior to or during plant growth stages with low to moderate N consumption for fertilisers with intermediate N delivery speed (F, LM, BM and PL), or for application during growth stages with high consumption rate of N for those with a rapid N delivery speed (PG and SN).

In summary, the results enabled classification of the organic fertilisers according to their N availability rate and N delivery speed, with some differences related to the properties of the soils. In this way, the fertilisers were classified as having a low rate of N availability, such as CO, medium rate of N availability, such as F, LM and BM, and a high rate of N availability, such as SN, PG and PL. Regarding N delivery speed, as an average of the results from the three soils, fertilisers PG and SN were categorised as having a rapid N delivery speed, while PL, BM and F had an intermediate N delivery speed, and LM and CO a slow N delivery speed. These results will be useful for organic producers, allowing for selection of N fertilisers for low, medium or high N requirements of the crop and for application at appropriate times within the crop cycle.

Disclosure statement

No potential conflict of interest was reported by the authors.

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