



Nitrification of leachates from manure composting under field conditions and their use in horticulture



Rafaela Cáceres^{a,*}, Albert Magrí^b, Oriol Marfà^a

^aIRTA, Research and Technology, Food and Agriculture, GIRO Unit, Cra. Cabrils km 2, 08348 Cabrils, Barcelona, Spain

^bIRSTEA, UR GERE, 17 Avenue de Cucillé, CS 64427, F-35044 Rennes, France

ARTICLE INFO

Article history:

Received 5 March 2015

Revised 30 June 2015

Accepted 22 July 2015

Available online 31 July 2015

Keywords:

Composting leachate

Nitrification

Nitrate

Lettuce

Nutrient uptake

Horticultural value

ABSTRACT

This work aimed to demonstrate the feasibility of nitrification applied to the treatment of leachates formed during composting of cattle and pig manure in order to promote their further use as liquid fertilizer in horticulture. Nitrification trials were successfully conducted in summer and winter seasons under Mediterranean climate conditions. Subsequently, effect of using the nitrified effluents as nutritive solution in the fertigation of lettuce (*Lactuca sativa* L.) was assessed in terms of productivity and nutrient uptake. Similar productivities were obtained when using the nitrified effluents and a standard nutritive solution. However, results also evidenced high nutrient uptake, which indicates that dosage should be adjusted to culture requirements.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The intensive livestock production substantially contributes to the economics of most developed countries (FAOSTAT, 2015). This productive sector is responsible for several issues which can affect the environment, including the generation of solid and liquid organic waste. Thus, appropriate waste management is needed to ensure the feasibility of this industry, and research can help in optimizing the agricultural use of such by-products. Concerning horticulture, potential targets to be studied are the organic amendment, fertilization, biostimulation against plant stress, correction of crop deficiencies, and production of growing media (Cáceres and Marfà, 2013; Marfà et al., 2009; Polo et al., 2006).

Particularly, manure processing through composting has extensively been investigated as a method for producing a value-added product (compost) for the recycling of organic matter and nutrients (Bernal et al., 2009). However, this treatment may involve formation of leachate because of several reasons: (i) the high moisture content of manure and the compression caused by the overlying material column (Mason et al., 2004); (ii) the application of water

to maintain the moisture content of the material that is being composted within an appropriate range (Cáceres et al., 2015; Tejada et al., 2008); and (iii) rainfall events – in uncovered composting systems – (Parkinson et al., 2004).

Composting leachate is a complex type of wastewater with high organic and mineral load (Gagnaire et al., 2011; Trujillo et al., 2006; Vázquez et al., 2013; Zhou et al., 2010). In composting facilities, leachate should be properly collected, stored, and managed in order to avoid negative environmental impacts such as aquatic eutrophication. Different alternatives have already been proposed for treating composting leachate targeting reduction of potentially polluting compounds (nutrients included), which are encompassed in two different sets of technologies; i.e., physicochemical technologies (Gagnaire et al., 2012; Liu and Lo, 2001; Trujillo et al., 2006), and biological technologies (Savage and Tyrrel, 2005; Tyrrel et al., 2008; Vázquez et al., 2013; Zhou et al., 2010). However, nutrient-rich wastewaters are broadly available for potential reuse as liquid fertilizer in crop production systems. Such reuse can reduce water consumption, the cost of the fertilizer, and the cost of leachate treatment/disposal, while resulting in environmental benefits (Chong et al., 2008). Several works have already described the agricultural use of this kind of effluents for crop production as foliar fertilizer for vegetables (Singh et al., 2010; Tejada et al., 2008), as well as fertilizer for cereals (Gutiérrez-Miceli et al., 2008), ornamental plants (Gils et al.,

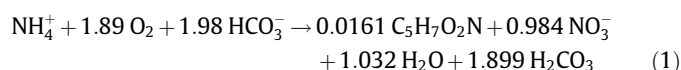
* Corresponding author.

E-mail addresses: rafaela.caceres@irta.cat (R. Cáceres), albert.magri@irstea.fr (A. Magrí), oriol.marfa@irta.cat (O. Marfà).

2005; Zhou et al., 2010), and trees (Justin et al., 2010). It was also reported that certain pre-conditioning treatment of the leachate (dilution, addition of microorganisms, storage, etc.) can favor such reuse (Gutiérrez-Miceli et al., 2008; Zhou et al., 2010). On the other hand, the main deterrents against using composting leachate in plant cultivation are nutrient imbalances, high salinity, and variability in the physiological response depending on the particular crop (Chong et al., 2008; Gils et al., 2005). Concerning the edible horticulture, the study of heavy metals and nutrient uptake as well as the translocation of undesirable substances to the plant is mandatory in order to assure not transferring these elements into the food chain (Al-Lahham et al., 2007).

Nitrogen (N) content in composting leachates is relatively high, and mainly in form of ammonium (NH_4^+). This is quite similar to the case of livestock slurries produced in cattle and swine farming (Flotats et al., 2009). High NH_4^+ concentrations may become phytotoxic in crop fertigation (Britto and Kronzucker, 2002; Bugarín et al., 1998). However, if NH_4^+ is previously transformed into nitrate (NO_3^-), the resulting effluent will be potentially usable for fertigation without toxicity risk for plants. In other words, such conversion will imply the production of a nitrogenous form easily assimilable by plants (Bugarín et al., 1998) while maintaining the fertilizing value of the leachate in terms of N units. In addition, by converting NH_4^+ into NO_3^- it is possible to prevent N loss due to volatilization during storage. This is because the NH_4^+ ion and ammonia (NH_3) are in chemical equilibrium depending on the pH and temperature (T) of the medium, and NH_3 is a volatile form of N (Ndegwa et al., 2008).

Nitrification is a two-step biological reaction catalyzed by autotrophic microorganisms. It consists in the oxidation of NH_4^+ into nitrite (NO_2^-) by ammonium-oxidizing bacteria (AOB), and subsequently, the oxidation of NO_2^- into NO_3^- by nitrite-oxidizing bacteria (NOB). The process basically requires appropriate T and pH, dissolved oxygen (DO) supply, and good substrate-to-biomass contact ratio. Since nitrifying microorganisms use inorganic carbon as carbon source, availability of organic matter, i.e., chemical oxygen demand (COD), is not necessary. According to the US EPA (1993), the complete nitrification reaction can be expressed as:



where $\text{C}_5\text{H}_7\text{O}_2\text{N}$ is the molecular expression for the nitrifying microorganisms. In accordance with this stoichiometry, bicarbonate (HCO_3^-) requirement during nitrification is equivalent to 1.98 mol HCO_3^- per mol of NH_4^+ oxidized (7.07 g $\text{CaCO}_3 \text{ g}^{-1} \text{NH}_4^+\text{-N}$). Such alkalinity consumption usually results in a decreased pH of the treated effluent. Technical feasibility of the nitrification process coupled to a denitrification strategy was already demonstrated in many studies targeting N removal from wastewaters derived from livestock activities (Deng et al., 2007; Magrí and Flotats, 2008; Magrí et al., 2012; Vázquez et al., 2013). However, nitrification alone can also become a chance for the valorization of these liquid effluents as nitrogenous fertilizer.

The objectives of this work are: (i) to assess the feasibility of nitrification applied to the treatment of leachates from cattle and swine manure composting, under Mediterranean climate conditions, in order to promote their subsequent use as liquid fertilizer, and (ii) to study the effect of this use in a horticultural culture of lettuce focusing on the evaluation of productivity and nutrient uptake.

2. Materials and methods

2.1. Composting leachates

Raw leachates were collected in a collective composting plant located in the Osona county (Catalonia, Spain), which processes manure coming from nearby cattle (C) and pig (P) farms. Cattle and pig manures were composted separately; therefore, two different kinds of leachate were obtained. The composting process was carried out in an open facility using windrows. Physicochemical characterization of the leachates (Table 1) evidenced basic pH and high electrical conductivity (EC) –particularly in the case of leachate P-. High $\text{NH}_4^+\text{-N}$ concentrations (600–900 mg N L^{-1}) make unfeasible direct leachate use in horticultural fertigation since it would lead to phytotoxicity issues. The carbon-to-nitrogen (C/N) ratio was estimated within the range of 3.2–4.6, which suggests potential coexistence of organic degradation and nitrification during aerobic treatment. Owing to the high level of total suspended solids (TSS) in leachate C (3.2 g L^{-1}), both leachates were subjected to gravity settling during 2 days and subsequent decanting prior to aerobic treatment.

2.2. Setup for leachates nitrification

Nitrification of the composting leachates as well as subsequent tests concerning use of the nitrified effluents in horticulture were both conducted in a glass greenhouse located in the experimental facilities of IRTA Cabrils (Catalonia, Spain; latitude $41^\circ 25' \text{N}$, longitude $2^\circ 23' \text{E}$, and height above sea level of 85 m) (Fig. 1).

The nitrification assays were carried out in three different time periods. The first trial “nitrification preliminary test” (Section 2.2.1) was performed using two reaction tanks. On the other hand, the second and third trials (concerning summer and winter seasons; Sections 2.2.2 and 2.2.3, respectively) were both performed using four reaction tanks. Thus, two different dilutions were considered at most for each type of leachate. All the tanks used were identical; i.e., tanks were made of polyethylene plastic, with cylindrical shape (56 cm diameter and 105 cm high) and total volume of 260 L. Tanks were empty at the beginning of a new

Table 1

Composition of the raw leachates coming from cattle manure composting (leachate C) and pig manure composting (leachate P). Results are referred to fresh sample except when otherwise indicated.

Parameter	Units	Leachate C	Leachate P
pH (1/5) ^a	–	8.4	8.4
EC (1/5) ^b	dS m^{-1}	2.80	4.05
TS	%	1.8	2.0
VS	%, dry weight	48.9	45.2
TSS	mg L^{-1}	3176	105
Org-N	mg L^{-1}	486	720
$\text{NH}_4^+\text{-N}$	mg L^{-1}	614	894
$\text{NO}_3^-\text{-N}$	mg L^{-1}	1.7	14.7
C/N ratio	g g^{-1}	4.6	3.2
P	mg L^{-1}	113	32
K	mg L^{-1}	3429	4734
Cl^-	mg L^{-1}	918	2133
Na	mg L^{-1}	630	1044
Ca	mg L^{-1}	614	102
Fe	mg L^{-1}	131	40
Mg	mg L^{-1}	153	58
S	mg L^{-1}	135	164
Cu	mg L^{-1}	2.14	0.38
Zn	mg L^{-1}	3.24	1.72

^a pH and EC were measured after dilution 1/5 (w/w; proportions referred to fresh leachate and distilled water, respectively).

^b EC: electrical conductivity, TS: total solids, VS: volatile solids, TSS: total suspended solids, Org-N: organic nitrogen.

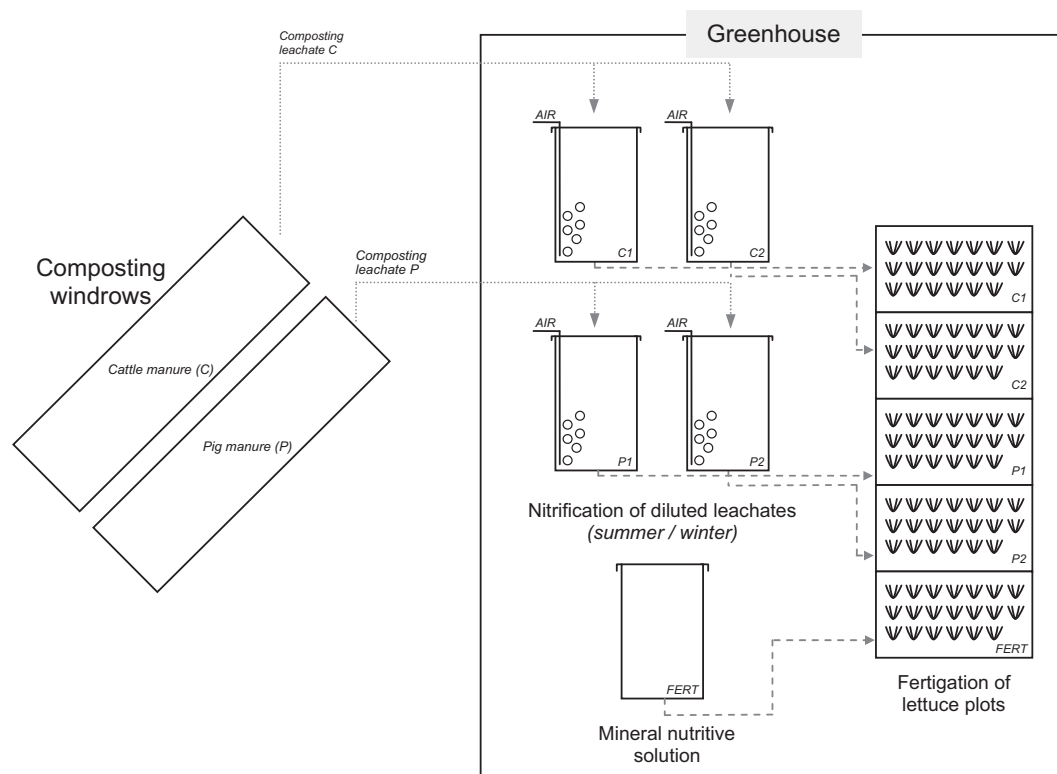


Fig. 1. Diagram of the experimental setup (summer and winter experiments).

experiment and then filled up with leachate and the corresponding amount of tap water needed for dilution (the working volume was set at 210 L). Tap water was added in order to decrease the salt content of the leachate, as it will be detailed later. The resulting solutions were aerated in batches during time enough to achieve complete oxidation of NH_4^+ into NO_3^- (tanks were not covered during this period). Aeration was provided through a turbobfan (power of 1.5 HP; nominal air flow rate of $150 \text{ m}^3 \text{ h}^{-1}$) placed inside of the greenhouse and connected to perforated plastic pipes (40 mm diameter) identically installed at the bottom of all tanks. This turbobfan was powered cyclically by means of a time programmer (model Junior™ MAX, Irritrol Systems, Riverside, CA, USA).

2.2.1. Nitrification preliminary test

This initial trial was carried out during the month of June and lasted ~1 month. It aimed to verify the nitrification potential of leachates from manure composting under the aforementioned conditions. Owing to the high NH_4^+ load and salinity of the leachates, dilution with water was considered in order to decrease the EC below a predefined threshold; i.e., $<3 \text{ dS m}^{-1}$, taking into account the tolerance to salinity of the lettuce plants (Ayers and Westcot, 1987). According to their composition (Table 1), leachate C needed lower dilution than leachate P; i.e., it should be noted that the EC of the raw leachates was measured after 1/5 (w/w) dilution, whereas measurement in the diluted leachates was carried out without pre-treating the sample. Thus, leachate C was diluted at a rate 1/7 whereas leachate P was diluted at a rate 1/15 (these proportions are referred to volumes of leachate and water, respectively). Diluted solutions were aerated until it was evidenced a substantial decrease of the NH_4^+ concentration and the consequent increase of the NO_3^- concentration. Aeration was supplied according to a progressive strategy; during the first week the turbobfan was powered 2 s per hour (low aeration rate – L), during the second week it was powered 10 s at intervals of 5 min (intermediate aeration rate – I),

and from the third week onwards it was powered 5 s per minute (high aeration rate – H).

2.2.2. Nitrification during the summer season

The nitrification trial in the summer season was carried out during the months of July and August. In order to decrease salinity, two dilutions of each leachate were performed. The targeted EC after dilution was $\sim 1\text{--}3 \text{ dS m}^{-1}$. Thus, dilution rates applied to leachate C were 1/7 (C1) and 1/10 (C2), whereas dilution rates applied to leachate P were 1/15 (P1) and 1/20 (P2) (these proportions are referred to volumes of leachate and water, respectively). The aeration rate in the nitrifying tanks was fixed as H (high aeration rate, according to the nitrification preliminary test) throughout the experimental period. *T* and DO were monitored regularly. Water samples were collected in order to follow the evolution of the N forms. Alternatively, water samples were also taken for the analysis of other parameters. Nitrification was considered to be completed when $\text{NH}_4^+\text{-N}$ was no longer present and the $\text{NO}_3^+\text{-N}$ concentration had increased significantly and maintained a constant value along time.

2.2.3. Nitrification during the winter season

The nitrification trial in the winter season was carried out during the months of November and December. The operating conditions were identical to those applied during the summer season. However, less analytical control was performed in this case. Monitored parameters were *T*, pH, EC, and the N forms.

2.3. Use of the nitrified effluents for the fertigation of lettuce

The nitrified effluents obtained in the summer and winter trials (procedure described in Sections 2.2.2 and 2.2.3) were used as nutritive solution for the cultivation of lettuce (*Lactuca sativa* L.). Seedlings of the cultivar Arena® (Vilmorin Clause & Cie, Paris,

France) type Batavia (Maravilla) were used. Lettuce was chosen because it is a vegetable that may accumulate relatively large amounts of nutrients and heavy metals (Jordão et al., 2007). Five fertigation treatments were applied (Table 2). Four of these treatments consisted on the continuous supply of the nitrified effluents available after the summer and winter season trials without any extra mineral addition (C1, C2, P1, and P2), and the fifth consisted on the continuous supply of a reference nutritive solution (FERT), which is similar to the procedure followed by Chong et al. (2008). As aforementioned, cultivation was performed inside a glass greenhouse. Individual benches (110 cm wide \times 150 cm long \times 40 cm high) filled with natural sandy-loam soil were used. Thus, five experimental plots were established, each one containing 20 lettuce plants (distance between plants was 30 cm). Lettuces were planted in November and were harvested three months later. Irrigation was provided by dripping at a nominal flow rate of 4 L h⁻¹, which resulted in a daily water supply of 200 mL per plant. Thus, the mean daily mineral N supplies for C1, C2, P1, P2, and FERT were 22.0, 15.9, 11.2, 6.5, and 21.5 mg N plant⁻¹ day⁻¹, respectively. According to Rincón Sánchez (2005), the recommended N supply through fertigation in commercial lettuce cropping in Mediterranean areas is 19.1 mg N plant⁻¹ day⁻¹.

2.4. Chemical analyses

2.4.1. Raw composting leachates

Analyses of the raw leachates (and water samples) were basically conducted according to the *Standard Methods for the Examination of Water and Wastewater* (APHA et al., 2005). The pH of fresh samples was measured using a specific probe (model ROSS Ultra[®] pH/ATC Triode, Thermo Scientific Orion, Nijkerk, Netherlands) plugged to an expandable ion analyzer (model EA 920, Orion Research Inc., Beverly, MA, USA). The EC (referred to 25 °C) of fresh samples was measured using a platinum conductivity cell (model 52 92, Crison Instruments S.A., Alella, Spain) plugged to a conductivity meter (model GLP 31, Crison Instruments S.A.). Total solids (TS) were determined after sample drying to constant weight at 105 °C, and volatile solids (VS) were determined after further ignition in a muffle furnace at 470 °C for 5 h. TSS were measured analogously to TS but after filtration through a glass fiber filter. Organic N (Org-N) was determined using the Kjeldahl method based on the digestion of a dry sample, distillation, and final measurement using a NH₄⁺ selective electrode. NH₄⁺ and NO₃⁻ were measured using selective electrodes (models 95–12 and 9307BNWP, respectively, Orion Research Inc.) plugged to the aforementioned expandable ion analyzer. The C/N ratio was estimated considering C as organic-C (0.58 * VS) and N as Org-N plus NH₄⁺-N (Magrí and Teira-Esmatges, 2015). Chloride (Cl⁻) was measured by ion chromatography (761 Compact IC, Metrohm AG, Herisau, Switzerland). Total phosphorus (P), potassium (K), sodium (Na), calcium (Ca), iron (Fe), magnesium (Mg), sulfur (S), copper (Cu), and zinc (Zn) were determined after micro-wave assisted acid digestion (using aqua regia) of a dry sample by an inductively coupled plasma-optical emission spectrometer (model ICP-OES-730-ES, Varian Inc., Santa Clara, CA, USA).

2.4.2. Water samples

Alkalinity was determined by acid titration to an end-point of pH 4.5 and expressed as CaCO₃. COD was determined by the dichromate method using a photometer (model CheckitDirect COD Vario, Tintometer GmbH, Lovibond Water Testing, Dortmund, Germany). Org-N was determined by digestion of a dry sample, distillation, and final titration. NO₃⁻ was determined using Merckoquant[®] nitrate test strips (Merck KGaA, Darmstadt, Germany) and a meter (model Nitrachek 404, KPG Products Ltd., Hove, UK) as frequently referred in the literature (Cossani et al., 2012; Sall and Vanclooster, 2009). In order to avoid interferences caused by NO₂⁻, several drops of amidosulfuric acid solution (10%) were added to the samples. In addition, the NO₃⁻ and NO₂⁻ content in some samples was determined using ion chromatography (761 Compact IC, Metrohm AG). Other analyses were carried out according to the procedures described in Section 2.4.1.

2.4.3. Nitrifying tanks

During the summer and winter season tests, *T* in the liquid bulk was measured using thermocouple sensors and recorded continuously (every 10 min) by means of a data logger (model 177, Testo AG, Lenzkirch, Germany). Greenhouse ambient *T* was also recorded using the same device. Data were analyzed off-line using mean and standard deviation. DO concentration was periodically measured using a specific probe (model 9009/2, Schott-Geräte, Mainz, Germany) plugged to a meter (model Consort Z 621, DESIN[®] Instruments, Turnhout, Belgium).

2.4.4. Lettuce plants

The relative amount of chlorophyll in leaves was determined by direct measurement using a soil plant analysis development (SPAD) chlorophyll meter (model SPAD-502, Konica Minolta, Osaka, Japan) as described by Gholizadeh et al. (2011). After plants harvesting, fresh and dry weights (60 °C for 72 h) were recorded. The NO₃⁻ content in leaves was determined using dry samples that were ground using a mortar grinder (model RM 100, Retsch GmbH, Haan, Germany). Processed samples were mixed with distilled water and the resulting solutions were shaken and filtered to obtain water extracts. NO₃⁻ was then measured using ion chromatography (Cáceres et al., 2012). Boron (B) and Manganese (Mn) were measured after micro-wave assisted acid digestion (using aqua regia) of a dry sample by ICP–OES. Other analyses were carried out according to the procedures described in Section 2.4.1. Eight replicates were considered for each treatment.

2.5. Statistical analyses

Data concerning productivity and nutrient uptake for the lettuce culture were subjected to analysis of variance (ANOVA) using the statistical package SAS v9.1 (SAS Institute, Cary, NC, USA). Averages of the quantitative factors under evaluation were compared by means of the Tukey's test.

Table 2

Physicochemical characteristics of the nitrified effluents (C1, C2, P1, and P2) and reference nutritive solution (FERT) used in the fertigation experiment with lettuce.

Nutritive solution	pH (–)	EC (dS m ⁻¹)	NH ₄ ⁺ (meq L ⁻¹)	NO ₃ ⁻ (meq L ⁻¹)	PO ₄ H ₂ ⁻ (meq L ⁻¹)	K ⁺ (meq L ⁻¹)	Cl ⁻ (meq L ⁻¹)	SO ₄ ²⁻ (meq L ⁻¹)	Na ⁺ (meq L ⁻¹)	Ca ²⁺ (meq L ⁻¹)	Mg ²⁺ (meq L ⁻¹)
C1	7.88	2.59	0.06	7.79	0.44	11.05	6.37	3.26	5.73	11.59	3.37
C2	7.87	2.00	0.03	5.64	0.32	8.06	5.61	3.39	4.88	10.85	3.00
P1	7.32	1.92	0.07	3.94	0.06	7.65	7.11	3.50	5.30	8.63	2.19
P2	7.92	1.28	0.04	2.28	0.05	5.85	6.27	3.55	4.67	8.68	2.15
FERT	6.51	1.83	0.81	6.86	1.30	2.54	3.58	4.67	2.63	5.00	3.88

3. Results and discussion

3.1. Leachates nitrification

3.1.1. Nitrification preliminary test

In order to ensure good aeration of the leachates, air flow rate in the nitrifying tanks was progressively increased from 0.20 L-air L-liquid⁻¹ h⁻¹ during the first week to 11.9 L-air L-liquid⁻¹ h⁻¹ during the second week, and finally to 29.8 L-air L-liquid⁻¹ h⁻¹ from the third week onwards. This increase in the air flow supply throughout the experimental period did not result in problems linked to foam formation. No significant changes in the N forms were observed during the first days of experiment (Fig. 2) although the environmental parameters were favorable for the nitrification process, i.e., $T = 25\text{--}30\text{ }^{\circ}\text{C}$ and $\text{pH} = 8.0\text{--}8.4$ (Grunditz and Dalhammar, 2001). After 9 days of experiment, $\text{NH}_4^+\text{-N}$ began to decline in leachate P. Similar trend was observed in leachate C after 12 days of experiment. However, $\text{NH}_4^+\text{-N}$ decrease was not proportional to $\text{NO}_2^-\text{-N}$ increase initially because of $\text{NO}_2^-\text{-N}$ accumulation, which was semi-quantitatively detected between days 10 and 25, using the Nitrachek® strip test. Finally, after 26 days of experiment all $\text{NO}_2^-\text{-N}$ was oxidized, and the final $\text{NO}_3^-\text{-N}$ concentration matched the initial $\text{NH}_4^+\text{-N}$ concentration (Fig. 2b).

Results obtained in this preliminary test showed the feasibility of promoting nitrification by means of intermittent aeration of diluted leachates without using nitrifying inoculum (only considering growth of indigenous microorganisms). The initially low aeration intensity applied could result in a longer time needed to complete nitrification. A priori, the basic pH (~ 8.0) and moderate EC ($\sim 2.5\text{ dS m}^{-1}$) of the aerated effluents did not seem limiting concerning a subsequent use in fertigation. The NO_2^- ion, which can become phytotoxic (Ezzine and Ghorbel, 2006), did not accumulate in the final effluent. Final $\text{NO}_3^-\text{-N}$ concentration in the nitrified effluents ($\sim 70\text{ mg N L}^{-1}$) was similar to the concentration in nutritive solutions regularly used for lettuce cropping in natural soils (Rincón Sánchez, 2005). Results obtained in this preliminary test suggested that the intermediate (I) or high (H) aeration rates were the best options for a fast oxidation of $\text{NH}_4^+\text{-N}$.

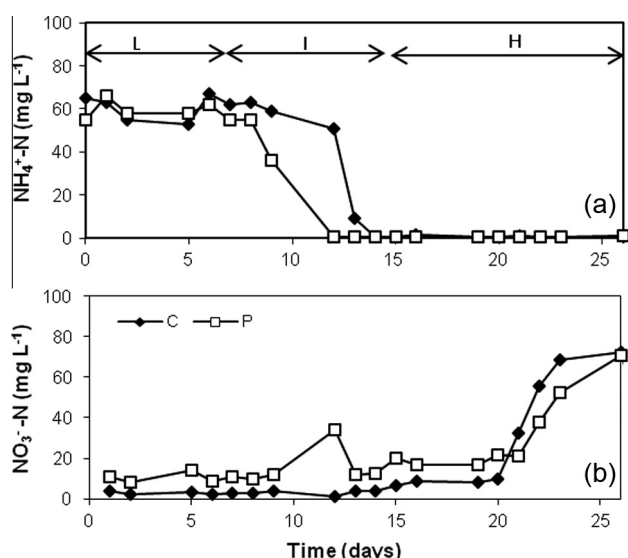


Fig. 2. Time course of (a) $\text{NH}_4^+\text{-N}$ and (b) $\text{NO}_3^-\text{-N}$ during the nitrification preliminary test. Aeration rate: L, low; I, intermediate; H, high.

3.1.2. Nitrification during the summer season

Four nitrification trials were performed involving use of two types of leachate (leachates C and P) and two dilution rates (rates 1 and 2); i.e., trials were named C1, C2, P1, and P2. According to the results obtained in the preliminary test (Section 3.1.1), air supply in the nitrifying tanks was fixed at the H rate (14.9 L-air L-liquid⁻¹ h⁻¹). Greenhouse ambient T was $27.5 \pm 4.5\text{ }^{\circ}\text{C}$. Otherwise, T in the liquid bulk ranged from $24\text{ }^{\circ}\text{C}$ to $36\text{ }^{\circ}\text{C}$ ($30.6 \pm 2.4\text{ }^{\circ}\text{C}$) throughout the experimental period, which is appropriate for the process performance. Indeed, nitrifying activity is expected to increase exponentially within this range but to stop above $35\text{ }^{\circ}\text{C}$ (Cáceres et al., 2006; Grunditz and Dalhammar, 2001). Owing to the dilution rates applied, initial $\text{NH}_4^+\text{-N}$ concentration was higher in those tests processing leachate C ($89\text{--}100\text{ mg N L}^{-1}$) than in those others processing leachate P ($38\text{--}57\text{ mg N L}^{-1}$) (Fig. 3 and Table 3).

Concerning the evolution of the N forms, the same pattern was evidenced in all the cases; i.e., $\text{NH}_4^+\text{-N}$ decrease was followed by a transient $\text{NO}_2^-\text{-N}$ increase, and finally by a complete oxidation into $\text{NO}_3^-\text{-N}$ (Fig. 3). The $\text{NH}_4^+\text{-N}$ conversion was faster in those cases with lower dilution, becoming complete at the third day for leachate C1 and at the fourth day for leachate P1 (Fig. 3). In those treatments with higher dilution, $\text{NH}_4^+\text{-N}$ was completely removed after 6–7 days. Finally, 5, 9, 14, and 19 days were needed to complete nitrification in the treatment of leachates P1, C1, C2, and P2, respectively (Fig. 3 and Table 3). The aforementioned transient accumulation of $\text{NO}_2^-\text{-N}$ could be caused by one, or a combination, of the following factors advantageous for AOB with respect to NOB: (i) low concentration of DO; (ii) mild T of operation; (iii) high concentration of free ammonia (FA, NH_3). Concerning DO, lower affinity for DO of NOB makes possible NO_2^- accumulation when concentration is below $1\text{--}1.5\text{ mg O}_2\text{ L}^{-1}$ (Ciudad et al., 2005; Magrí and Flotats, 2008; Zeng et al., 2009). However, in this experiment DO was not limiting and even reached values higher than $5\text{ mg O}_2\text{ L}^{-1}$ once NH_4^+ was totally consumed (air supply could be thus optimized according to a predefined DO set-point). Concerning T , the NOB activity has been reported to be slower than the AOB activity at temperatures higher than $15\text{--}20\text{ }^{\circ}\text{C}$. Otherwise, the reverse is true at lower temperatures (Hellinga et al., 1998). In this experiment T of the liquid bulk fluctuated daily with an average value of $30.6 \pm 2.4\text{ }^{\circ}\text{C}$. Finally, concerning FA, concentrations higher than $0.8\text{ mg NH}_3\text{-N L}^{-1}$ may completely inhibit the oxidation of NO_2^- into NO_3^- (Anthonisen et al., 1976). In our experiment even higher levels were reached; e.g., 60 mg N L^{-1} of total ammonium at $30\text{ }^{\circ}\text{C}$ and $\text{pH } 8.4$ are equivalent to 10 mg N L^{-1} of FA.

The Org-N content in the aerated leachates increased throughout the experiment on dry weight basis (Fig. 4) which could be partly justified due to the growth of the microbial community (heterotrophs and nitrifying bacteria) during the aeration process. In this regard, the biomass yields (conversion into biomass of substrates) proposed by Henze et al. (1999) are $0.62\text{ g COD-formed g}^{-1}\text{ COD-oxidized}$ for heterotrophs and $0.24\text{ g COD-formed g}^{-1}\text{ N-oxidized}$ for nitrifying bacteria, respectively. Assuming the composition of the new cells as $\text{C}_5\text{H}_7\text{O}_2\text{N}$ (Eq. (1)), a mass equivalence ratio of $0.086\text{ g Org-N g}^{-1}\text{ COD-formed}$ should be applicable.

The N mass balance considering initial and final composition of the nitrified leachates is presented in Fig. 5, which shows that the N concentration was mainly conserved in the aqueous solutions at the end of the nitrification period. In spite of this fact, lack of gas emissions monitoring prevented experimental quantification of NH_3 volatilization. The authors hypothesize that some N losses could have occurred during the experiment according to the initial $\text{NH}_4^+\text{-N}$, pH , T and also due to the aeration of the leachates. However, such losses are not evidenced in the mass balance because of the slight evaporation of water, and consequent concentration of dissolved compounds, observed at the end of the

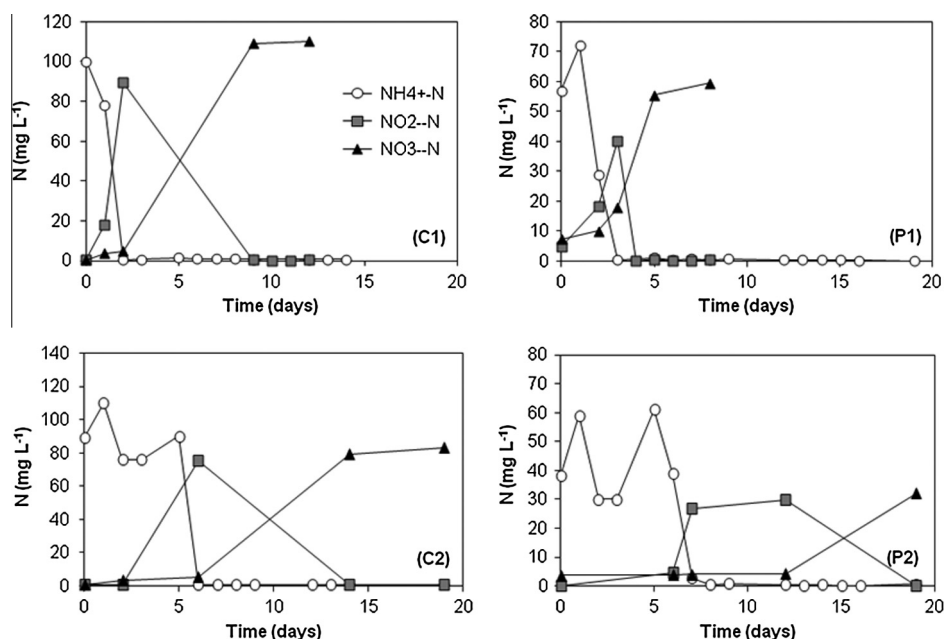


Fig. 3. Time course of $\text{NH}_4^+\text{-N}$ (o), $\text{NO}_2^-\text{-N}$ (■), and $\text{NO}_3^-\text{-N}$ (▲) during the nitrification study in the summer season.

Table 3

Initial (I) and final (F) composition of the nitrified leachates (summer season).

Parameter ^a	Stage	C1	C2	P1	P2
pH (–)	I	8.10	8.16	8.07	8.02
	F	7.88	7.87	7.32	7.92
EC (dS m ^{–1})	I	2.62	1.91	1.78	1.28
	F	2.59	2.00	1.92	1.28
Org-N (mg L ^{–1})	I	69.6	41.6	54.8	17.7
	F	102.0	37.2	84.4	37.5
$\text{NH}_4^+\text{-N}$ (mg L ^{–1})	I	100.0	89.0	57.0	38.0
	F	0.9	0.4	1.0	0.6
$\text{NO}_2^-\text{-N}$ (mg L ^{–1})	I	0.7	0.6	4.6	0.2
	F	0.5	0.4	0.5	0.2
$\text{NO}_3^-\text{-N}$ (mg L ^{–1})	I	0.4	0.5	7.2	3.7
	F	109.1	79.0	55.5	32.0
Alkalinity (mg CaCO ₃ L ^{–1})	I	900	630	440	310
	F	410	220	150	150
COD (mg O ₂ L ^{–1})	I	1647	1158	941	550
	F	1242	1248	725	463
Elapsed time (days)		9	14	5	19
T ± SD (°C)		30.8 ± 2.5	30.5 ± 2.3	30.4 ± 2.4	30.4 ± 2.6

^a EC: electrical conductivity, Org-N: organic nitrogen, COD: chemical oxygen demand, T: temperature, SD: standard deviation.

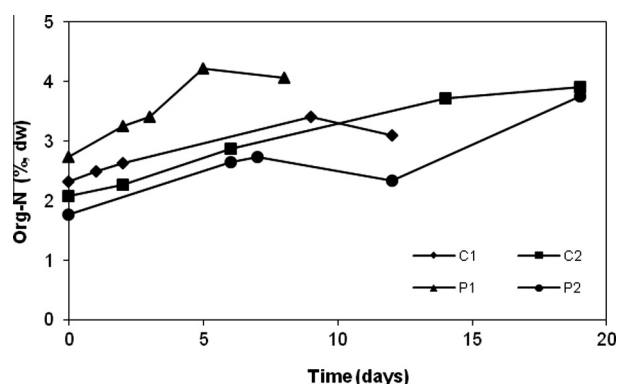


Fig. 4. Time course of Org-N (expressed on dry weight basis) during the nitrification study in the summer season. Org-N, organic nitrogen.

experimental period. Thus, the experimental management of this research could have partly masked the dynamics of the N species, although is clearly demonstrated that $\text{NH}_4^+\text{-N}$ was mainly transformed into $\text{NO}_3^-\text{-N}$ throughout the nitrification process. Fig. 3 helps supporting this evidence, including experimental points at different times. On the other hand, $\text{NO}_2^-\text{-N}$ concentrations were negligible at the end of the experiment (Fig. 5).

Nitrification involved a decrease in the pH and final values were within the range of 7.3–7.9, which are not limiting for the nitrification process. Indeed, the degree of acidification is expected to depend on the initial NH_4^+ concentration and the buffer capacity of the medium (Chen et al., 2006; Daumer et al., 2007). The on-line evaluation of the pH trend can also be used in order to identify the NH_4^+ oxidation end-point (Gu et al., 2012). High alkalinities were measured in the diluted leachates (310–900 mg CaCO₃ L^{–1}) whereas final values ranged from 150 to 410 mg CaCO₃ L^{–1} (Fig. 6), which implies significant consumptions during treatment (Fig. 6a). Thus, ratios between alkalinity consumption and $\text{NO}_3^-\text{-N}$ production on concentration basis were within 4.5 and 6.0 g CaCO₃ g^{–1} $\text{NO}_3^-\text{-N}$ (Fig. 6b), which is below the ratio derived from Eq. (1) (7.07 g CaCO₃ g^{–1} $\text{NH}_4^+\text{-N}$) and can be partly attributed to the slight evaporation of water observed at the end of the

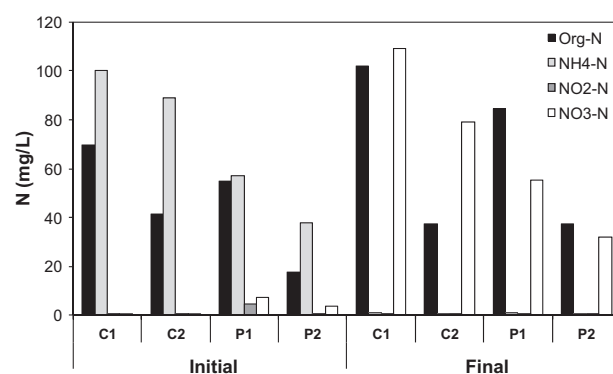


Fig. 5. Nitrogen mass balance considering initial and final composition of the nitrified leachates.

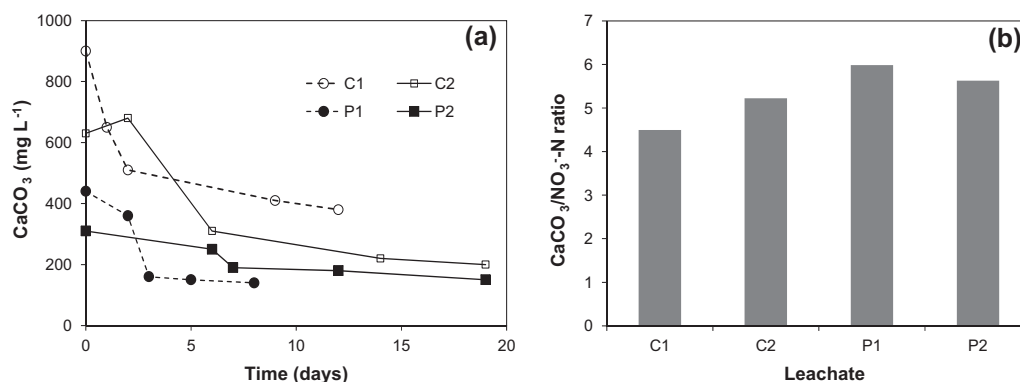


Fig. 6. (a) Time course of alkalinity (CaCO₃) during the nitrification study in the summer season and (b) ratios between alkalinity consumed and NO₃-N produced (on concentration basis).

treatment. Concerning salinity, although it may limit nitrification at high levels (Sudarmo et al., 2011), the dilution rates applied in this study allowed to work within a suitable range, with EC values ranging from 1.28 to 2.62 dS m⁻¹.

Initial COD values for the diluted leachates ranged from 550 to 1647 mg O₂ L⁻¹ (Table 3). However, such COD was demonstrated to be partly non-biodegradable (removal efficiencies were lower than 25%). Under aerobic conditions, the heterotrophic microorganisms responsible for the biodegradation of organic compounds compete for oxygen and space with the nitrifying bacteria, which are known to be less competitive. In this regard, heterotrophic bacteria have maximum growth rates ~6 times higher, and yields ~3 times higher, than nitrifying bacteria (Henze et al., 1999). Thus, a pre-treatment of the leachates in order to promote energetic valorization of the organic load as proposed by Romero et al. (2013) may be also advantageous for a subsequent treatment of nitrification, since it would help in reducing competence of heterotrophic bacteria. In addition, lower availability of biodegradable organic-C could prevent potential undesired events of heterotrophic denitrification during intermittent aeration, and the consequent loss of N as dinitrogen gas (N₂).

Regarding the use of the nitrified effluents for the fertigation of lettuce, final NO₃⁻ concentration was appropriate in all the treatments except for P2 -i.e., lowest concentration- (Rincón Sánchez, 2005) (Table 2), with the highest concentrations measured in the aerated leachates C1 and C2. NH₄⁺ was almost inexistent, and presence of Org-N could benefit fertilization because of its slow release (Table 3). The pH and EC of the nitrified effluents were relatively high in comparison to the values of the standard nutritive solution FERT. Alkalinity (including bicarbonates) was lower than 420 mg CaCO₃ L⁻¹, and thus similar to the values proposed by Gils et al. (2005) in nutritive solutions with moderate acidity. Concerning other elements, concentrations could be lower (e.g., P), similar (e.g., Mg), or higher (e.g., K, Na, Cl, and Ca) than in the reference solution FERT (Table 2) depending on the particular case. Thus, globally the nitrified effluents presented an unbalanced composition, which is otherwise usual when working with wastewater reuse in fertigation (Chong et al., 2008).

3.1.3. Nitrification during the winter season

The same trials performed during the summer season were repeated during the winter season in order to evaluate effect of *T*. Greenhouse ambient *T* throughout the experimental period was 15.1 ± 5.2 °C whereas *T* in the liquid bulk averaged 15.8 ± 2.8 °C. Indeed, *T* in the liquid phase ranged from 15 °C to 20 °C (17.9 ± 1.9 °C) during the first 34 days but then progressively decreased to 10–15 °C (13.3 ± 1.4 °C) during the following 21 days. An extension of the time needed for completing NH₄⁺-N oxidation

was observed, since it was not achieved until 14–24 days after starting up the process (data not shown). In addition, significant formation of NO₃⁻-N (above 45 mg N L⁻¹) took 40 days or longer (treatment of leachate P1 was the fastest). Thus, time needed to achieve complete nitrification during the winter season was at least double than during the summer season (Section 3.1.2). However, nitrification was still feasible at ~10 °C in agreement to previous works reported in the literature (Guo et al., 2010; Kim et al., 2006; Weon et al., 2004). Final pH of the nitrified effluents was 8.1–8.2 concerning leachate C and 7.0–7.3 concerning leachate P, whereas final EC of the nitrified effluents was similar to the values reached in the summer season (1.17–3.31 dS m⁻¹).

3.2. Use of the nitrified effluents for the fertigation of lettuce

3.2.1. Productivity and quality parameters

Results showed that lettuces fertirrigated with nitrified effluent C2 weighted more on fresh basis (fw) than lettuces fertirrigated with nitrified effluents P1 and P2. However, no significant differences were observed with respect to those lettuces cropped using nitrified effluent C1 and the reference nutritive solution FERT (Table 4). On the other hand, no significant differences were found between treatments when dry weight was evaluated. Thus, it can be concluded that differences in the chemical composition of the nutritive solutions used for fertigation did not affect lettuce productivity.

NO₃⁻ content in the nitrified effluents appeared to be high enough to compete with other ions such as Cl⁻ for uptake

Table 4

Biometric parameters concerning lettuce plants depending on the nutritive solution supplied (C1, C2, P1, P2, and FERT).

Nutritive solution	Fresh weight (fw) ^a (g)	Dry weight (dw) (g)	SPAD ^b measurements (–)	NO ₃ ⁻ (mg kg ⁻¹ , fw)	NO ₃ ⁻ (mg kg ⁻¹ , dw)
C1	422 ab	14.5	24.2 ab	389 a	11,322 a
C2	531 a	17.3	25.3 b	320 b	9848 a
P1	389 b	14.1	27.9 ab	217 ab	5996 bc
P2	399 b	14.6	29.8 a	210 c	5743 c
FERT	480 ab	15.4	26.9 ab	314 ab	9811 ab
<i>p</i>	0.016	0.2653	0.0031	0.0002	0.0002
Significance	**	ns	***	***	***

^a Values are means of eight replicates; Values in a column followed by the same letter are not significantly different ($p \leq 0.05$); Symbols used in ANOVA are: *p* = probability, ns = not significant at $p \leq 0.05$, ** = significant at $p \leq 0.02$, *** = significant at $p \leq 0.01$.

^b SPAD: soil plant analysis development.

(Gils et al., 2005) (Table 2). On the other hand, NO_3^- content in leaves was higher when applying nitrified effluents from leachate C than when applying nitrified effluents from leachate P (Table 4). Thus, there was a clear correlation between the NO_3^- content in the fertilizing solution and the NO_3^- content in leaves (Fig. 7). This correspondence had already been reported in previous studies (Cáceres et al., 2012; Khoshgoftarmanesh et al., 2011; Pavlou et al., 2007). Despite this fact, NO_3^- concentrations measured in lettuce leaves were much lower than the threshold of $3500 \text{ mg NO}_3^- \text{ kg}^{-1} \text{ fw}$ laid down by the Regulation 1881/2006 (Commission of the European Communities, 2006). It is well-known that NO_3^- plays a key role in the function and nutrition of plants and that accumulation can occur in leaf crops such as lettuce and spinach. It has also been demonstrated that NO_3^- accumulation in lettuce leaves can occur under continuous supply of NO_3^- because of the low activity of the nitrate reductase enzyme in the latter stages of the plant growth (Pinto et al., 2014b). In this regard, NO_3^- is relatively non-toxic for humans, but this may not be the same case of other derived compounds and metabolites; e.g., NO_2^- , nitric oxide, and nitrosamines (Pinto et al., 2014b).

Statistical analysis of the SPAD measurements only showed significant differences between plants irrigated with the nitrified effluents C2 and P2 (Table 4). The SPAD measurements inversely correlated with the NO_3^- content in leaves. Higher NO_3^- uptakes could have promoted higher expansions of the lettuce leaves in terms of leaf area index (LAI) that would have resulted in decreased chlorophyll contents. Indeed, it has already been described that as N supply is increased, a higher proportion of

assimilated N can be stored in leaves without affecting the photosynthesis (Marschner, 1986). In addition, the SPAD measurements positively correlated with the Fe content in leaves ($R=0.6707$; Tables 4 and 5), which was described to occur under availability of oxidized forms of this metal. In this regard, the leachates could have contained chelate that would have ameliorated the Fe uptake by complexation (He et al., 2015; López, 2007).

3.2.2. Mineral content in leaves

Significant differences between treatments were found when assessing the mineral content in leaves (Table 5). Total N and P were significantly higher in the case of using the nutritive solution FERT. The lower pH of this nutritive solution (Table 2) may have influenced in the higher P uptake. However, this fact did not result in a higher productivity. Thus, from an agronomic point of view, this extra nutrient uptake could be considered as a luxury uptake. The K content in leaves was not significantly different in case of using nutritive solutions such as nitrified effluents C1, C2, and FERT, but it was lower when using the nitrified effluent P2. In general terms, the K content in leaves was very high for all the treatments, and clearly above the N levels, as previously reported elsewhere (Baslam et al., 2013; Maroto Borrego et al., 2000).

Results concerning Na were also meaningful. Concentration in the nitrified effluents doubled concentration in the solution FERT (Table 2). According to this, those treatments that entailed use of nitrified effluents resulted in Na contents in leaves clearly higher than in case of using the reference solution (Table 5), and which can be considered as very high with respect to other studies (Baslam et al., 2013). Content in leaves of other micronutrients such as Fe, Cu, and Zn could be also moderately high with respect to reference levels detailed elsewhere (Baslam et al., 2013; Maroto Borrego et al., 2000; Pinto et al., 2014a), but for Fe and Zn were still below the toxic thresholds stated by Rincón Sánchez (2005). It is interesting to highlight that the highest Cu and Zn uptakes were measured when using the nitrified effluent P1 (Table 5). These heavy metals may be present at significant levels in pig manure and may translocate into the plant when the pH of the soil is relatively low (Loué, 1988). In this regard, in a field study performed with tomato plants irrigated with water containing high levels of potentially toxic elements, Al-Lahham et al. (2007) observed how at high concentrations these elements may translocate into the fruit at a ratio dependent on the cultivar used. Thus, it could be advisable to extend the use as nutritive solution of nitrified leachates formed during manure composting to non-alimentary crops such as ornamental crops in order to minimize human health risks. In some works dealing with the use of composting leachates as foliar fertilizers, significant enhancements (with respect to exclusive water supply) were evidenced in terms of plant productivity.

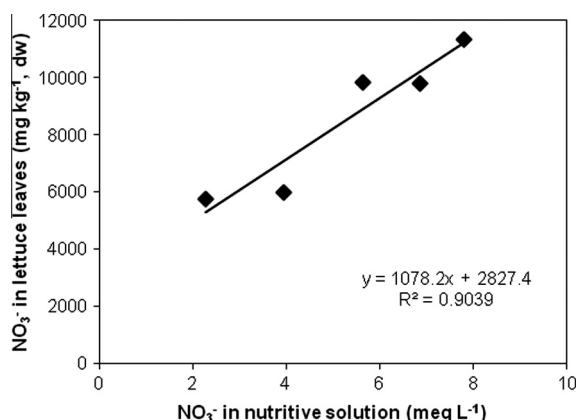


Fig. 7. Correlation between the NO_3^- concentration in the nutritive solutions and the NO_3^- content in the lettuce leaves (on dry weight basis).

Table 5

Mineral composition of the lettuce leaves depending on the nutritive solution supplied (C1, C2, P1, P2, and FERT). Results expressed on dry weight basis.

Parameter	C1	C2	P1	P2	FERT	p	s
N (%)	3.50 b	3.40 b	3.38 b	3.33 b	3.72 a	<0.0001	***
P (%)	0.41 bc	0.48 b	0.41 bc	0.40 c	0.80 a	<0.0001	***
K (%)	10.18 ab	10.44 a	8.19 c	9.03 bc	9.55 ab	<0.0001	***
Na (%)	0.86 ab	0.89 ab	0.95 a	0.81 b	0.46 c	<0.0001	***
Ca (%)	1.41 ab	1.27 bc	1.26 bc	1.23 c	1.58 a	<0.0001	***
Mg (%)	0.36 b	0.29 c	0.42 a	0.39 ab	0.36 b	<0.0001	***
S (%)	0.32	0.31	0.30	0.30	0.32	0.4862	ns
B (mg kg ⁻¹)	58.2 ab	62.5 a	45.4 c	47.5 c	50.5 bc	<0.0001	***
Fe (mg kg ⁻¹)	317	448	422	483	345	0.8973	ns
Mn (mg kg ⁻¹)	77.6 a	57.1 bc	73.2 ab	76.0 a	43.0 c	<0.0001	***
Cu (mg kg ⁻¹)	23.5 b	21.8 b	65.2 a	19.4 b	22.1 b	0.0114	**
Zn (mg kg ⁻¹)	140 ab	115 b	230 a	143 ab	82 b	0.0026	***

Values are means of eight replicates; Values in a row followed by the same letter are not significantly different ($p \leq 0.05$); Symbols used in ANOVA are: p = probability, s = significance, ns = not significant at $p \leq 0.05$, ** = significant at $p \leq 0.02$, *** = significant at $p \leq 0.01$.

However, authors mainly attribute such positive physiological response not to the high nutrient concentration, but to the presence of humic substances (Tejada et al., 2008). It has been demonstrated that humic acids may increase the number of roots thereby stimulating nutrient uptake and plant growth and development (Singh et al., 2010).

4. Conclusions

Technical feasibility of nitrifying leachates formed during composting of cattle and pig manure was demonstrated. Nitrification was particularly tested under greenhouse conditions, using diluted leachates, and without supplying any external microbial inoculum. Process rates were significantly higher during the summer season because of the higher temperatures. Hence, longer processing periods were necessary during the winter season in order to achieve complete nitrification. Extended aeration avoided NO_2^- accumulation in the treated effluents. Final NO_3^- concentrations were similar to those in standard nutritive solutions used in lettuce cropping.

The nitrified effluents were suitable for lettuce fertigation despite showing certain imbalances with respect to a synthetic mineral solution. The productivity gained using these nitrified effluents was similar to the productivity gained using a standard nutritive solution. Results also demonstrated the need to be cautious in the use of nitrified effluents for the fertigation of edible crops due to nutrient uptake. Under the irrigation flow rate provided, the higher the NO_3^- content in the nutritive solution used, the higher the NO_3^- content in the lettuce leaves. Thus, dosage should be adjusted to meet culture requirements.

The experiments presented here are a first approach on the reuse of composting leachates for horticulture irrigation. Further research should be carried out before promoting a full-scale application. This study provides new scientific information concerning the valorization of leachates from cattle and pig manure composting as liquid fertilizers. Nitrification of the leachates was accomplished by taking into account field conditions in Mediterranean climate regime, and after appropriate dilution. The use of the nitrified effluents for the fertigation of lettuce was satisfactorily assessed, and results demonstrated the feasibility of using this type of nutrient-rich wastewater in horticultural fertigation. Overall, this may imply a lower dependence on synthetic fertilizers, and represents an opportunity for the environmentally friendly reuse of the leachates formed in manure composting plants while promoting lowering water consumption and practical application of nutrient recycling strategies in agriculture.

Acknowledgements

This study was supported by the Spanish National Institute for Agricultural and Food Research and Technology (INIA; Project RTA2010-00112-00-00) of the Ministry of Economy and Competitiveness. Authors thank Anna Puerta for the technical assistance throughout the project.

References

Al-Lahham, O., El Assi, N.M., Fayyad, M., 2007. Translocation of heavy metals to tomato (*Solanum lycopersicum* L.) fruit irrigated with treated wastewater. *Sci. Hortic.* 113, 250–254.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of nitrification by ammonia and nitrous acid. *J. Water Pollut. Control Fed.* 48, 835–852.

APHA, AWWA, WEF, 2005. Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

Ayers, R.S., Westcot, D.W., 1987. La Calidad del Agua en la Agricultura. Estudio FAO: Riego y Drenaje, 29, Rev. 1. Roma, Italy (in Spanish).

Baslam, M., Morales, F., Garmendia, I., Goicoechea, N., 2013. Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. *Sci. Hortic.* 151, 103–111.

Bernal, M.P., Alburquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* 100, 5444–5453.

Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. *J. Plant Physiol.* 159, 567–584.

Bugarín, M.R., Baca, C.G.A., Martínez, H.J., Tirado, T.J.L., Martínez, G.A., 1998. Amonio/nitrato y concentración iónica total de la solución nutritiva en crisantemo. I. Crecimiento y floración. *Terra Latinoamericana* 16, 113–124 (in Spanish).

Cáceres, R., Coromina, N., Malińska, K., Marfà, O., 2015. Evolution of process control parameters during extended co-composting of green waste and solid fraction of cattle slurry to obtain growing media. *Bioresour. Technol.* 179, 398–406.

Cáceres, R., Flotats, X., Marfà, O., 2006. Changes in the chemical and physicochemical properties of the solid fraction of cattle slurry during composting using different aeration strategies. *Waste Manage.* 26, 1081–1091.

Cáceres, R., Marfà, O., 2013. Diagnosis of the fertility of compost-based growing media: method comparison and monitoring in pot plant cultivation. *Sci. Hortic.* 164, 213–220.

Cáceres, R., Turet, J., Marfà, O., 2012. Evaluación agronómica de composts de estiércoles como abono orgánico en un cultivo de lechuga en suelo natural. In: Barral Silva, M.T., Díaz-Raviña, M., Devesa-Rey, R., Paradelo Núñez, R. (Eds.), *Avances en la Investigación sobre Compost: Materias Primas, Procesos, Calidad y Usos*. Andavira Editora S.L., Santiago de Compostela, Spain, pp. 523–531 (in Spanish).

Chen, S., Ling, J., Blancheton, J.-P., 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquac. Eng.* 34, 179–197.

Chong, C., Purvis, P., Lumis, G., Holbein, B.E., Voroney, R.P., Zhou, H., Liu, H.-W., Alam, M.Z., 2008. Using mushroom farm and anaerobic digestion wastewaters as supplemental fertilizer sources for growing container nursery stock in a closed system. *Bioresour. Technol.* 99, 2050–2060.

Ciudad, G., Rubilar, O., Muñoz, P., Ruiz, G., Chamy, R., Vergara, C., Jeison, D., 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological removal process. *Process Biochem.* 40, 1715–1719.

Commission of the European Communities, 2006. Regulation No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union* L364, pp. 5–24.

Cossani, C.M., Slafer, G.A., Savin, R., 2012. Nitrogen and water use efficiencies of wheat and barley under a Mediterranean environment in Catalonia. *Field Crop. Res.* 128, 109–118.

Daumer, M.L., Béline, F., Guizieu, F., Sperandio, M., 2007. Effect of nitrification on phosphorus dissolving in a piggery effluent treated by a sequencing batch reactor. *Biosyst. Eng.* 96, 551–557.

Deng, L., Cai, C., Chen, Z., 2007. The treatment of pig slurry by a full-scale anaerobic-adding raw wastewater-intermittent aeration process. *Biosyst. Eng.* 98, 327–334.

Ezzine, M., Ghorbel, M.H., 2006. Physiological and biochemical responses resulting from nitrite accumulation in tomato (*Lycopersicon esculentum* Mill. cv. Ibiza F1). *J. Plant Physiol.* 163, 1032–1039.

FAOSTAT, 2015. Food and Agriculture Organization of the United Nations. Internet: <<http://faostat3.fao.org/home/E>> (accessed February 2015).

Flotats, X., Bonmati, A., Fernández, B., Magrí, A., 2009. Manure treatment technologies: on-farm versus centralized strategies. NE Spain as case study. *Bioresour. Technol.* 100, 5519–5526.

Gagnaire, J., Chapon, L., Moulin, P., Marrot, B., 2012. Physico-chemical treatment applied to compost liquor: feasibility study. *J. Ind. Eng. Chem.* 18, 1522–1528.

Gagnaire, J., Wang, X.Y., Chapon, L., Moulin, P., Marrot, B., 2011. Kinetic study of compost liquor nitrification. *Water Sci. Technol.* 63, 868–876.

Gholizadeh, A., Amin, M.S.M., Anuar, A.R., Aimrun, W., Saberioon, M.M., 2011. Temporal variability of SPAD chlorophyll meter readings and its relationship to total nitrogen in leaves within a Malaysian paddy field. *Aust. J. Basic Appl. Sci.* 5, 236–245.

Gils, J., Chong, C., Lumis, G., 2005. Response of container-grown ninebark to crude and nutrient-enriched recirculating compost leachates. *HortScience* 40, 1507–1512.

Grunditz, C., Dalhammar, G., 2001. Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. *Water Res.* 35, 433–440.

Gu, S., Wang, S., Yang, Q., Yang, P., Peng, Y., 2012. Start up partial nitrification at low temperature with a real-time control strategy based on blower frequency and pH. *Bioresour. Technol.* 112, 34–41.

Guo, J., Peng, Y., Huang, H., Wang, S., Ge, S., Zhang, J., Wang, Z., 2010. Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. *J. Hazard. Mater.* 179, 471–479.

Gutiérrez-Miceli, F.A., García-Gómez, R.C., Rincón Rosales, R., Abud-Archila, M., Oliva Llavén, M.A., Guillen Cruz, M.J., Dendooven, L., 2008. Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* (L.) Moench) using vermicompost leachate. *Bioresour. Technol.* 99, 6174–6180.

He, X.-S., Xi, B.-D., Zhang, Z.-Y., Gao, R.-T., Tan, W.-B., Cui, D.-Y., Yuan, Y., 2015. Composition, removal, redox, and metal complexation properties of dissolved organic nitrogen in composting leachates. *J. Hazard. Mater.* 283, 227–233.

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M., Heijnen, J.J., 1998. The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water. *Water Sci. Technol.* 37 (9), 135–142.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M.C., Marais, G.v.R., van Loosdrecht, M.C.M., 1999. Activated sludge model No. 2D, ASM2D. *Water Sci. Technol.* 39 (1), 165–182.

- Jordão, C.P., Fialho, L.L., Neves, J.C.L., Cecon, P.R., Mendonça, E.S., Fontes, R.L.F., 2007. Reduction of heavy metal contents in liquid effluents by vermicomposts and the use of the metal-enriched vermicomposts in lettuce cultivation. *Bioresour. Technol.* 98, 2800–2813.
- Justin, M.Z., Pajk, N., Zupanc, V., Zupančič, M., 2010. Phytoremediation of landfill leachate and compost wastewater by irrigation of *Populus* and *Salix*: biomass and growth response. *Waste Manage.* 30, 1032–1042.
- Khoshgoftarmanesh, A.H., Hosseini, F., Afyuni, M., 2011. Nickel supplementation effect on the growth, urease activity and urea and nitrate concentrations in lettuce supplied with different nitrogen sources. *Sci. Hortic.* 130, 381–385.
- Kim, D.-J., Lee, D.-I., Keller, J., 2006. Effect of temperature and free ammonia on nitrification and nitrite accumulation in landfill leachate and analysis of its nitrifying bacterial community by FISH. *Bioresour. Technol.* 97, 459–468.
- Liu, C.-H., Lo, K.V., 2001. Ammonia removal from compost leachate using zeolite. II. A study using continuous flow packed columns. *J. Environ. Sci. Health Part B – Pestic. Contam. Agric. Wastes* 36, 667–675.
- López, M., 2007. Effect of EDDHA in Soil Solution and FeEDDHA in the Soybean Rhizosphere. MSc. Thesis, Wageningen, Netherlands.
- Loué, A., 1988. Los Microelementos en Agricultura. Mundi-Prensa, Madrid, Spain (in Spanish).
- Magrí, A., Flotats, X., 2008. Modelling of biological nitrogen removal from the liquid fraction of pig slurry in a sequencing batch reactor. *Biosyst. Eng.* 101, 239–259.
- Magrí, A., Teira-Esmatges, M.R., 2015. Assessment of a composting process for the treatment of beef cattle manure. *J. Environ. Sci. Health Part B – Pestic. Contam. Agric. Wastes* 50, 432–440.
- Magrí, A., Vanotti, M.B., Szögi, A.A., Cantrell, K.B., 2012. Partial nitrification of swine wastewater in view of its coupling with the anammox process. *J. Environ. Qual.* 41, 1989–2000.
- Marfà, O., Cáceres, R., Polo, J., Ródenas, J., 2009. Animal protein hydrolysate as a biostimulant for transplanted strawberry plants subjected to cold stress. *Acta Horticulturae (ISHS)* 842, 315–318.
- Maroto Borrego, J.V., Gómez, A.M., Baixauli Soria, C., 2000. La Lechuga y la Escarola. Fundación Caja Rural Valencia, Mundi-Prensa, Madrid, Spain (in Spanish).
- Marschner, H., 1986. Mineral Nutrition of Higher Plants, first ed. Academic Press, London, UK.
- Mason, I.G., Mollah, M.S., Zhong, M.F., Manderson, G.J., 2004. Composting high moisture content bovine manure using passive aeration. *Compost Sci. Util.* 12, 249–267.
- Ndegwa, P.M., Hristov, A.N., Arogo, J., Sheffield, R.E., 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosyst. Eng.* 100, 453–469.
- Parkinson, R., Gibbs, P., Burchett, S., Misselbrook, T., 2004. Effect of turning regime and seasonal weather conditions on nitrogen and phosphorus losses during aerobic composting of cattle manure. *Bioresour. Technol.* 91, 171–178.
- Pavlou, G.C., Ehalotis, C.D., Kavvadias, V.A., 2007. Effect of organic and inorganic fertilizers applied during successive crop seasons on growth and nitrate accumulation in lettuce. *Sci. Hortic.* 111, 319–325.
- Pinto, E., Almeida, A.A., Aguiar, A.A.R.M., Ferreira, I.M.P.L.V.O., 2014a. Changes in macrominerals, trace elements and pigments content during lettuce (*Lactuca sativa* L.) growth: influence of soil composition. *Food Chem.* 152, 603–611.
- Pinto, E., Fidalgo, F., Teixeira, J., Aguiar, A.A., Ferreira, I.M.P.L.V.O., 2014b. Influence of the temporal and spatial variation of nitrate reductase, glutamine synthetase and soil composition in the N species content in lettuce (*Lactuca sativa*). *Plant Sci.* 219–220, 35–41.
- Polo, J., Barroso, R., Ródenas, J., Azcón-Bieto, J., Cáceres, R., Marfà, O., 2006. Porcine hemoglobin hydrolysate as a biostimulant for lettuce plants subjected to conditions of thermal stress. *HortTechnology* 16, 483–487.
- Rincón Sánchez, L., 2005. La Fertirrigación de la Lechuga Iceberg. Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Murcia, Spain (in Spanish).
- Romero, C., Ramos, P., Costa, C., Márquez, M.C., 2013. Raw and digested municipal waste compost leachate as potential fertilizer: comparison with a commercial fertilizer. *J. Clean. Prod.* 59, 73–78.
- Sall, M., Vanclooster, M., 2009. Assessing the well water pollution problem by nitrates in the small scale farming systems of the Niayes region, Senegal. *Agric. Water Manage.* 96, 1360–1368.
- Savage, A.J., Tyrrel, S.F., 2005. Compost liquor bioremediation using waste materials as biofiltration media. *Bioresour. Technol.* 96, 557–564.
- Singh, R., Gupta, R.K., Patil, R.T., Sharma, R.R., Asrey, R., Kumar, A., Jangra, K.K., 2010. Sequential foliar application of vermicompost leachates improves marketable fruit yield and quality of strawberry (*Fragaria x ananassa* Duch.). *Sci. Hortic.* 124, 34–39.
- Sudarno, U., Winter, J., Gallert, C., 2011. Effect of varying salinity, temperature, ammonia and nitrous acid concentrations on nitrification of saline wastewater in fixed-bed reactors. *Bioresour. Technol.* 102, 5665–5673.
- Tejada, M., Gonzalez, J.L., Hernandez, M.T., Garcia, C., 2008. Agricultural use of leachates obtained from two different vermicomposting processes. *Bioresour. Technol.* 99, 6228–6232.
- Trujillo, D., Font, X., Sánchez, A., 2006. Use of Fenton reaction for the treatment of leachate from composting of different wastes. *J. Hazard. Mater.* B138, 201–204.
- Tyrrel, S.F., Seymour, I., Harris, J.A., 2008. Bioremediation of leachate from a green waste composting facility using waste-derived filter media. *Bioresour. Technol.* 99, 7657–7664.
- US EPA, 1993. Manual Nitrogen Control, EPA/625/R-93/010. U.S. Environmental Protection Agency, Office of Research and Development, Center for Environmental Research Information, Risk Reduction Engineering Laboratory, Cincinnati, OH, USA.
- Vázquez, M.A., de la Varga, D., Plana, R., Soto, M., 2013. Vertical flow constructed wetland treating high strength wastewater from swine slurry composting. *Ecol. Eng.* 50, 37–43.
- Weon, S.-Y., Lee, S.-I., Koopman, B., 2004. Effect of temperature and dissolved oxygen on biological nitrification at high ammonia concentrations. *Environ. Technol.* 25, 1211–1219.
- Zeng, W., Zhang, Y., Li, L., Peng, Y.-Z., Wang, S.-Y., 2009. Control and optimization of nitrifying communities for nitrification from domestic wastewater at room temperatures. *Enzyme Microb. Technol.* 45, 226–232.
- Zhou, C., Wang, R., Zhang, Y., 2010. Fertilizer efficiency and environmental risk of irrigating Impatiens with composting leachate in decentralized solid waste management. *Waste Manage.* 30, 1000–1005.