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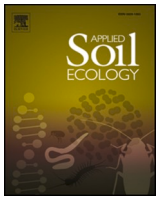

Dynamics of soil nitrogen and N-cycling-related genes following the application of biobased fertilizers

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ABSTRACT

Biobased fertilizers recovered from animal manure are sustainable substitutes for synthetic mineral nitrogen (N) fertilizers, showing high potential to minimize environmental pollution while maintaining nutrient supply. This study investigated the response of maize (*Zea mays* L.) and soil microbes to the application of biobased fertilizers, i.e., pig manure (PM), the liquid fraction of digestate (LFD), and evaporator concentrate (EVA) in replacement of calcium ammonium nitrate (CAN) in a 56-day pot experiment. Apart from maize plant growth and soil chemical properties, the abundance of N-cycling-related genes was determined by destructive sampling on days 8, 16, and 56 after fertilization. The detected gene copies of bacterial and archaeal *amoA* in the soil were significantly increased by N fertilization. Relatively high NH_4^+-N concentrations (37.5–62.5 mg kg^{-1} soil dry weight) applied in this experiment may have promoted gaseous N losses via nitrifier nitrification and nitrifier denitrification shortly (8–16 days) after fertilization. Consequently, net N loss was observed in all the fertilized treatments, however, biobased fertilizers resulted in lower N loss as compared to CAN. The presence of maize plants also reduced the N loss, probably driven by the continuous NO_3^--N uptake which reduced the N source for denitrification. Overall, the application of PM and LFD revealed no significant difference with CAN regarding either plant growth or soil biochemical properties. Whereas the EVA application resulted in lower biomass and nutrient uptake in the young maize plant compared to other treatments, probably attributed to salt stress due to the imbalanced ratio of N and Na in this product.

1. Introduction

A worldwide concern arises from the conflict between the increasing demand for nitrogen (N) fertilizers for food production and the surplus of manure N from intensified livestock production. Recycling manure N as fertilizer is regarded as an important strategy in re-connecting crop production and livestock husbandry and maintaining the sustainability of the agricultural systems. According to the Nitrate Directive (91/676/EEC), the application of manure and manure-derived fertilizing products is limited to 170 kg N ha^{-1} per year in the EU nitrate vulnerable zones (NVZs) to prevent pollution of groundwater. The theory behind lays in the observation that, compared to synthetic mineral N fertilizers, these manure-based products usually provide part of N in organic form, which is not readily available for the crop but could be released as mineral N at a time when the crop demand for N is low, and consequently increases the risks for N leaching. To this end, the Joint Research Center (JRC) of the European Commission has proposed the RENURE (REcovered Nitrogen from manURE) criteria to promote the safe use of manure-derived biobased fertilizers above the threshold established for NVZs (i.e., to reduce the cost on mineral fertilizer). The RENURE materials should have a ratio of mineral N over total N (minN/TN) higher than 90 % or a ratio of total organic C over total N (TOC/TN) lower than 3. Meanwhile,

Abbreviations: CAN, calcium ammonium nitrate; PM, pig manure; LFD, liquid fraction of digestate; EVA, evaporator concentrate; RENURE, recoveried nitrogen from manure; WFPS, water-filled pore space; NVZ, nitrate vulunerable zone; NUE, nitrogen use efficiency; RO, reverse osmosis; PCA, principal component analysis; SMN, soil mineral nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen..

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the concentrations of copper (Cu) and zinc (Zn) should be lower than the limits of 300 and 800 mg kg^{-1} dry matter, respectively.

Accordingly, most of the RENURE materials are liquids that need to be incorporated into the soil immediately after application to reduce the risk of ammonia emission (Huygens et al., 2020), e.g., by fertilizer distributors/applicators equipped with deep plowing shovels or injection wheels. For arable crops like maize (*Zea mays* L.), split fertilizer doses are usually applied at different growing stages, e.g., basal fertilization before sowing and top-dressing at V6–V10 (Fernandez et al., 2020). However top-dressing with these biobased fertilizers is not feasible yet, due to a lack of specific fertilizer distributors/applicators which can be used in the rows of maize plants (>30 cm) without damaging the plants. Moreover, split fertilization requires extra labor as compared to one-time fertilization at preplant. Therefore, currently in Flanders (Belgium), most of the biobased fertilizers are applied at full dose before planting to meet the full fertilizer requirement of the crop.

Though preplant fertilization has been recognized as the most common and feasible practice in arable farming (Montealegre et al., 2019), it was reported to cause lower nitrogen use efficiency (NUE) than split fertilization (Aula et al., 2021; Melaj et al., 2003), as a result of higher N losses during the early season when the young plants show limited N uptake. Biobased fertilizers supply a part of the N in organic form, which is less prone than mineral N to leaching and volatilization upon fertilization (Reganold and Wachter, 2016; Wei et al., 2020b). Additionally, the organic N in biobased fertilizers is usually associated with labile or recalcitrant C, which could be used by soil microbes as energy sources (Soong et al., 2020) and thus affect the N-cycling pathways (Cui et al., 2016; Ren et al., 2020; Tatti et al., 2013; Yang et al., 2017). However, the effect of biobased fertilizers may vary due to the various C and N composition of biobased fertilizers leading to completely different N availability and release patterns after soil application (Cavalli et al., 2018; Reuland et al., 2022; Hendriks et al., 2021). For example, a lower abundance of the bacterial *amoA* gene was reported in digestate-amended soil compared to the application of synthetic mineral fertilizer (i.e., liquid urea ammonium nitrate), indicating a lower nitrification potential which resulted in a smaller NO_3^--N pool and lower risk of nitrate leaching and denitrification (Ren et al., 2020). On another hand, when synthetic mineral fertilizer (urea and ammonium nitrate, respectively) was replaced by pig manure (Cui et al., 2016) and compost (Tatti et al., 2013), a higher abundance of the nitrite reductase genes *nirS* and *nirK* and the N_2O reductase gene *nosZ* were found, indicating the higher potential of denitrification.

Among the biobased fertilizers derived from animal manure, digestate and the liquid fraction of digestate (LFD) were characterized (Reuland et al., 2022; Sigurnjak et al., 2017; Zilio et al., 2022) with a higher N availability and a lower TOC/TN ratio than the raw manure, thus showing better compliance with the RENURE criteria. However, it is also reported that biobased fertilizers derived from manure may also pose a risk of nutrient imbalance, especially regarding excess supply of phosphorus (P), potassium (K), sulfur (S) or sodium (Na) when the crop N requirement is to be fully met (Fangueiro et al., 2018). This issue may be more pronounced in concentrated products recovered from evaporation or membrane processes which are usually high in electrical conductivity (Brienza et al., 2022; Hendriks et al., 2021; Luo et al., 2022; Saju et al., 2022) due to the removal of water (Chiumenti et al., 2013). To date, little has been demonstrated for these biobased fertilizers regarding the potential impact of imbalanced nutrients on plant growth and soil chemical and biological properties.

To fill the knowledge gap, this study investigated the performance of biobased fertilizers upon preplant fertilization on young maize plants and the N-cycling-related genes in soil, with the main focus on their potential value to completely replace synthetic mineral N fertilizers. Three biobased fertilizers, i.e., pig manure (PM), the liquid fraction of digestate (LFD) derived from anaerobic digestion, evaporation concentrate (EVA) recovered from the evaporation process, were compared against a zero-N control and a synthetic reference using calcium ammonium nitrate (CAN). The target genes, bacterial and archaeal *amoA*, *nirK* and *nosZ*, were selected based on recent literature reporting the abundances of these genes as suitable proxies for nitrification and denitrification processes, for example, Yang et al. (2017) observed significant correlations between the abundance of bacterial and archaeal *amoA* and *nirK* genes with the soil organic and NO_3^--N concentrations; (Han et al., 2020) found higher *nosZ* gene abundance under the long-term organic farming; the observations by Grunert et al. (2019) at lab conditions and by Zilio et al. (2020) at field conditions also reflected the significant impact of fertilizer type on the relative abundance of bacterial and archaeal *amoA*, showing their potential to map the N cycling pathways within the plant-soil system under BBFs and synthetic fertilizers. Apart from N cycling, the supplies of P, K, Na, and S by biobased fertilizers and their impact on plant growth and soil microbial communities were also evaluated. We hypothesized that (i) replacing synthetic fertilizers with biobased fertilizers could meet the N demand of young maize plants without increasing the short-term N loss after preplant fertilization; (ii) when N is not the limiting factor, the imbalanced nutrients provided by biobased fertilizers may negatively affect the activities of soil microbes and thus the N cycling pathways.

2. Materials and methods

2.1. Collection and characterization of biobased fertilizers

The biobased products were obtained from a biogas plant (Waterleau NewEnergy) located in Ieper, Belgium (50°51 N, 2°53E). The biogas plant has a yearly capacity of treating 12,000 m^3 of biowaste, consisting of 45 % pig manure (PM) and 55 % biological waste streams (grain waste, potato waste, glycerin and sludge from industrial wastewater treatment). Fig. 1 shows the process flow diagram of this nutrient recovery cascade. Briefly, the feedstock is mixed and heated to 40 °C before the 30-day anaerobic digestion which is followed by a 10-day post-digestion. The generated digestate is hygienized (1 h 70 °C) and separated by a decanter centrifuge. The resulting liquid fraction of digestate (LFD) goes into a biological aerobic water treatment for a small removal of organic matter. In the next step (evaporator), NH_3 is transferred to the gas phase and condenses with 1.5 % of the water vapor to form N-rich ammonia water (AW), while the remaining non-volatile nutrients are concentrated into an evaporator concentrate (EVA), representing 14 % of the fed volume. The rest of the water vapor (process water) goes through a reverse osmosis membrane unit. Sulphuric acid (H_2SO_4) is added to the effluent of the evaporator to enhance the removal of residual ammonium from the process water. This results in purified water which is dischargeable, and a reverse osmosis concentrate which is circulated to the aerobic treatment process.

The collected biobased fertilizers (PM, LFD, and EVA) were stored in the fridge at 4 °C for physicochemical characterization. Dry weight (DW) content was determined as the residual weight after 24 h drying at 105 °C. Organic matter (OM) was measured after incineration (loss on ignition) of the samples for 4 h at 550 °C in a muffle furnace (Nabertherm, Lilienthal, Lower Saxony, Germany). Electrical conductivity (EC) and pH were determined potentiometrically using a conductivity electrode (Orion Star 212A, Thermo Fisher Scientific, Waltham, MA, USA) and a pH meter (Orion Star 211A, Thermo Fisher Scientific, Waltham, MA, USA), respectively. Contents of total N (TN), total C (TC), and inorganic C (IC) were determined using a CN analyzer (Skalar Analytical BV, Breda, North Brabant, the Netherlands). The total organic C (TOC) was then calculated as the difference between TC and IC. The concentrations of mineral N (NH_4^+-N and NO_3^--N) were analyzed from 1 M KCl extract (1:10 *w/v*) using a continuous flow auto-analyzer (Chemlab System 4, Skalar, Breda, North Brabant, the Netherlands). The concentrations of macronutrients (P, K, S, and Na) and heavy metals (Zn and Cu) in the tested products were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista MPX, Varian, Inc., Palo Alto, CA, USA) from extracts after a closed microwave (CEM MARS 5,

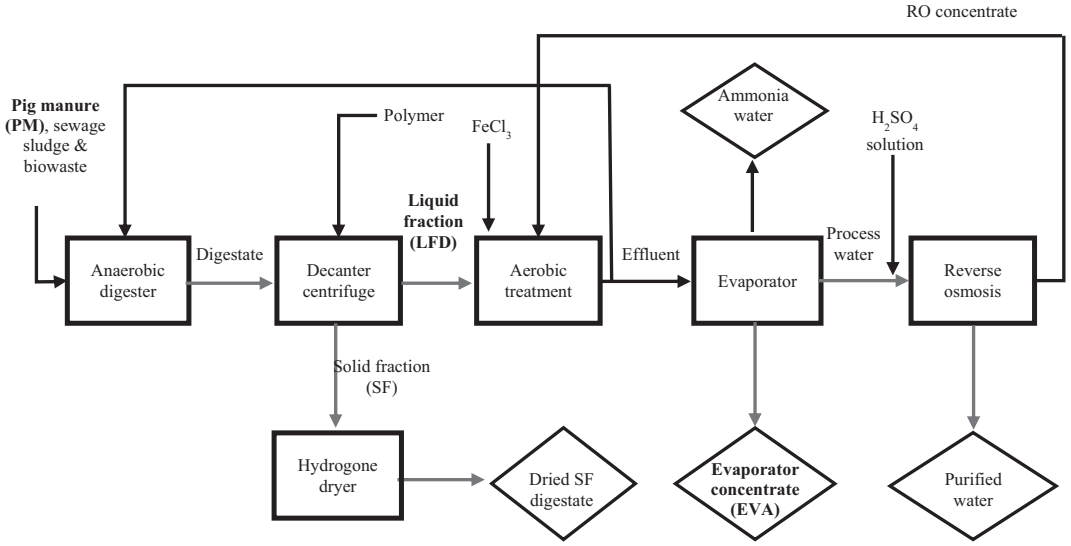

Fig. 1. Process flow diagram of the nutrient recovery system at the site of Waterleau NewEnergy (adapted from Brienza et al., 2022). Products indicated in bold were tested in this study.

Drogenbos, Flemish Brabant, Belgium) digestion using 6.5 % HNO_3.

The characteristics of the tested products are presented in Table 1.

2.2. Soil sampling and characterization

The soil used for this experiment was collected from the surface (0–30 cm) of an experimental farm of Ghent University in Bottelare (Belgium), containing 45 % of sand, 5 % of clay, and 50 % of silt, and texture is classified as silty-loam (USDA texture triangle). The soil was air-dried and sieved through a 2-mm mesh before characterization. A subsample of 10 g air-dried soil was extracted with 50 ml deionized water after 16 h equilibrium. Then the pH-H_2O and $EC_{1:5}$ were determined from the extracts using a conductivity electrode (Orion Star 212A, Thermo Fisher Scientific, Waltham, MA, USA) and a pH meter (Orion Star 211A, Thermo Fisher Scientific, Waltham, MA, USA), respectively. The determination of OM, TC, TN and mineral N in soil samples followed the same method as in Section 2.1. Three sub-samples were extracted by the ammonium lactate (pH = 3.75) at a ratio of 1:10 w/v for measurements of extractable nutrients (P, K, S, and Na) on inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista MPX, Varian, Inc., Palo Alto, CA, USA).

The characteristics of the tested soil are listed in Table 2:

2.3. Pot experiment set-up and sampling

The pot experiment consisted of seven treatments: (1) non-planted control with no N fertilizer applied (CON-NP), (2) planted control with no N fertilizer applied (CON-P), (3) non-planted synthetic reference using calcium ammonium nitrate as N fertilizer (CAN-NP), (4) planted synthetic reference using calcium ammonium nitrate as N fertilizer (CAN-P), (5) planted biobased treatments using pig manure (PM-P), (6) planted biobased treatment using the liquid fraction of digestate (LFD-P), and (7) planted biobased treatment using evaporator concentrate (EVA-P). The applied total N amount was 62.5 mg N kg^{-1} soil on a dry matter basis, equivalent to 262 kg N ha^{-1} (calculated on a surface area basis, taking into account the surface area of the pots). Accordingly, the highest P application was supplied by PM (20.8 mg P kg^{-1} soil DW, equals 87 kg P ha^{-1}), while the highest K application was supplied by EVA (75.5 mg K kg^{-1} soil DW, equals 316 kg K ha^{-1}). These P and K rates were compensated in all treatments with triple superphosphate (46 % P_2O_5) and potassium sulfate (consisting of 30 % K_2O, 10 % MgO, and 42 % SO_3) fertilizers.

Table 1
Characterization of biobased fertilizers (n = 3) on a fresh weight (FW) basis.

Parameters	Unit	Pig manure	Liquid fraction of digestate	Evaporator concentrate
pH	/	7.97 ± 0.01	9.04 ± 0.01	6.64 ± 0.03
Electrical conductivity (EC)	dS m^{-1}	24.7 ± 0.6	32.3 ± 0.2	109.0 ± 1.6
Dry weight (DW)	g kg^{-1}	105.3 ± 0.7	21.0 ± 0.3	182.6 ± 1.7
Organic matter (OM)	g kg^{-1}	77.0 ± 0.8	11.6 ± 0.10	108.8 ± 0.4
Total carbon (TC)	g kg^{-1}	23.5 ± 1.6	4.00 ± 0.03	35.3 ± 1.0
Total organic carbon (TOC)	g kg^{-1}	20.1 ± 1.7	2.54 ± 0.04	35.2 ± 1.2
Total nitrogen (TN)	g kg^{-1}	6.77 ± 0.30	4.75 ± 0.11	17.2 ± 0.2
NH_4^+-N	g kg^{-1}	4.52 ± 0.32	3.72 ± 0.07	9.82 ± 0.42
NO_3^--N	g kg^{-1}	<0.002	<0.002	<0.002
Mineral N/TN		0.67	0.78	0.57
TOC/TN	/	2.97	0.53	2.05
Total phosphorus (P)	g kg^{-1}	2.23 ± 0.06	0.16 ± 0.00	1.39 ± 0.05
Total potassium (K)	g kg^{-1}	4.34 ± 0.14	2.26 ± 0.00	18.7 ± 0.5
Total sulfur (S)	g kg^{-1}	0.91 ± 0.01	1.88 ± 0.04	18.4 ± 0.1
Total sodium (Na)	g kg^{-1}	1.42 ± 0.08	1.46 ± 0.03	7.21 ± 0.13
Total zinc (Zn)	mg kg^{-1}	92.3 ± 1.3	2.14 ± 0.23	32.0 ± 1.8
Total copper (Cu)	mg kg^{-1}	31.3 ± 0.7	0.43 ± 0.13	9.23 ± 0.32

Table 2
Chemical characteristics of the tested soil (in dry weight basis, $n = 3$).

	pH-H_2O	$EC_{1:5}$	OM	TC	TN	NH_4^+-N	NO_3^--N	Extractable P	Extractable K	Extractable S	Extractable Na
Unit	/	μS cm^{-1}	%	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Value	7.26	82	3.3	10.4	0.92	1.8	22.8	220	151	29.2	17.9

Fertilizers were mixed homogenously with 3 kg of air-dried soil and filled into squared pots (13 × 13 × 18 cm). Three maize (*Zea mays* L.) seeds were sown into each pot of the treatments CON-P, CAN-P, PM-P, LFD-P, and EVA-P. After germination (5 days), the maize plants were thinned to one plant per pot. The temperature was controlled at 20.8 ± 1.5 °C during the daytime (14 h) and 16.4 ± 0.8 °C during the night (10 h). The pots were weighed every 2–3 days, and deionized water was added to maintain the moisture content constant at a level equivalent to about 60 % of water-filled pore space (WFPS) (taking into account the bulk density of the soil in the pots, namely 1.4 g cm^3), as calculated based on the following equation:

$$WFPS\% = (GWC \times BD)/(1–(BD/PD)) \times 100 \quad (1)$$

where *GWC* is the gravimetric water content (g water g $soil^{-1}$), *BD* is the dry bulk density (Mg m^{-3}), and *PD* is the particle density (Mg m^{-3}). Any water leaching from the pots was recovered on a plate at the bottom and returned to the soil surface, i.e. N loss through leaching can be considered negligible.

Destructive sampling of 28 pots (seven treatments in quadruplicates) was done by removing intact pots on day 8, day 16, and day 56. At each sampling, soil samples were collected and analyzed following the same methods as described in Section 2.2. The maize plant shoots were cut with a knife from the soil surface. Roots were separated from the soil and washed with deionized water. All shoot and root samples were dried at 65 °C in a forced-draft oven until the DW was constant. Then, the dried biomass was homogeneously ground for TC and TN measurements using a CN analyzer (Skalar Analytical BV, Breda, North Brabant, the Netherland). The concentrations of P, K, S, and Na in plant samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista MPX, Varian, Inc., Palo Alto, CA, USA) from extracts after a closed microwave (CEM MARS 5, Drogenbos, Flemish Brabant, Belgium) digestion using 6.5 % HNO_3.

2.4. Microbial biomass C and N measurements

Determination of soil microbial biomass C and N (MBC, MBN) was performed by the fumigation–extraction method according to Vance et al. (1987) and Brookes et al. (1985). For each pot, a subsample of 60 g of fresh soil was collected and divided into two aliquots: one was fumigated with ethanol-free chloroform for 24 h at 20 °C and the other unfumigated. Both sets were extracted with 60 ml 0.1 M K_2SO_4. The organic C and extractable N in the extracts were determined in the fumigated and unfumigated samples using a TOC analyzer (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan). The MBC and MBN were calculated from the difference of organic C and extractable N between the fumigated and un-fumigated samples divided by the extraction coefficients $k_{EC} = 0.45$ (Joergensen, 1996) and $k_{EN} = 0.54$ (Brookes et al., 1985), respectively.

2.5. DNA extraction and target gene quantification by qPCR

Total DNA extractions were carried out on a quantity of soil equal to 0.5 g per sample. For total DNA extraction, the DNeasy® PowerSoil® Kit (Qiagen GmbH, Hilden, Germany) was used. The yield and purity (A260/A280 and A260/A230) of the extracted DNA were then quantified using the Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Quantitative PCR (qPCR) was performed on real-time 7900HT (Applied Biosystems, Thermo Fisher Scientific, Wilmington, USA) using SyberGreen technology, in a final volume of 10 μl. The sequences of the primers used are reported in Supplemental material Table S1. As templates for the standard curves, amplicons for each target gene were cloned into purified plasmids (pGem-T; Promega Corp.) and inserted into E.coli JM101 by electroporation. Knowing the size of the vector (3015 bp) and those for each insert (data from the literature, Table S1), and measuring the plasmid DNA concentration, the number of copies per ng of DNA and the corresponding amounts to be used for each of the qPCR calibration curves were calculated. The number of gene copies per gram of soil in each sample was then calculated by comparing the output of the qPCR with the calibration curve for the corresponding gene. Data analysis was performed using SDS v2.1 software (Applied Biosystems, Thermo Fisher Scientific, Wilmington, USA).

2.6. Calculations and statistical analyses

The N availability of each treatment was evaluated by the concentration of plant-available N (PAN), i.e. the sum of soil mineral N (SMN = NH_4^+-N + NO_3^--N) and plant N uptake at each sampling moment (day 8, day 16 and day 56 after fertilization). On day 0, since the SMN was only measured before fertilization, the calculation of the PAN also included the mineral N applied by fertilizers. Then the PAN balance was calculated as follows:

$$\begin{aligned} \text{PAN balance} &= \text{PAN on day } i – \text{PAN on day } 0 \\ &= \text{SMN on day } i + \text{plant N uptake on day } i \\ &\quad –(\text{SMN on day } 0 + \text{fertilizer mineral N}), \end{aligned} \quad (2)$$

where $i = 8$, 16 or 56. A positive balance indicates net N mineralization, while a negative balance indicates net N immobilization or N loss.

Statistical analyses were performed using SPSS statistical software (version 27.0; SPSS Inc., Chicago, IL). Due to the combination of planted and unplanted treatments in this experiment setup, the normality of residuals and the homogeneity of variance were not statistically supported. Therefore, the independent-samples Kruskal-Wallis analysis was used to determine the effect of time (day 8, 16 and 56) and the applied fertilizers (CAN, PM, LFD, EVA) on the maize biomass and N uptake, the PAN balance, the soil properties and the functional N-cycling-related genes. When significant differences ($p < 0.05$) between means were observed, additional post hoc assessment was performed using the Dunn test. These differences are indicated by the different lower or upper case letters.

To investigate the interrelations between fertilization, plant and soil parameters, Pearson's correlations were determined based on the observations of day 56 for the planted treatments CON-P, CAN-P, PM-P, LFD-P and EVA-P. A principal component analysis (PCA) was performed to evaluate the relationships between the soil physical (i.e. WFPS), chemical (pH-H_2O, $EC_{1:5}$, NH_4^+-N and NO_3^--N) and biological properties (abundance of bacterial and archaeal *amoA, nirK* and *nosZ* genes) during the experimental period. The result (Supplemental material Fig. S1) showed a distinct effect of time separating the soil properties on day 56 from those observed on day 16 and day 8, which is more significant than the effect of fertilizer treatments. Therefore, a PCA mainly based on the soil properties observed on day 56 was further performed to evaluate the fertilizer effect on soil physical (i.e. WFPS), chemical (i.e. pH-H_2O, $EC_{1:5}$, SMN, and soil Na) and biological properties (i.e. abundance of bacterial and archaeal *amoA, nirK* and *nosZ* genes).

3. Results

3.1. Maize shoot and root biomass and nutrient uptake

By the end of this experiment (day 56), PM-P, LFD-P and EVA-P resulted in lower DW of maize shoot than CON-P and CAN-P, with a significantly lower value of shoot DW observed in EVA-P, but the root DW revealed no significant difference when comparing between the planted treatments (Table2). However, there was a significantly higher root Na concentration (Data not shown) while a significantly lower uptake of N, P, K and S in the whole maize plant (including shoot and root) in EVA-P as compared to CON-P, CAN-P and PM-P.

3.2. Dynamics of soil chemical and biological properties

The application of CAN, PM, LFD and EVA led to significantly higher NH_4^+-N concentrations in soil by day 8, as compared to CON with no N fertilizer applied (Fig. 2 a). By day 16, the concentration of NH_4^+-N in CON-NP, CON-P and EVA-P declined to a level under the detection limit. While in CAN-NP, CAN-P and LFD-P, it remained over 10 mg N kg^{-1} soil DW, which reduced to a concentration < 5 mg N kg^{-1} soil DW by day 56. Fertilization also led to higher concentrations of NO_3^--N by day 8 (for CAN-NP and CAN-P) and day 16 (for PM-P and EVA-P), respectively, as compared to those in CON-NP and CON-P (Fig. 2 d).

The abundance of four N-cycling-related genes was determined in this study: the archaeal and bacterial *amoA* as molecular markers for ammonia-oxidizing communities, the nitrite reductase gene *nirK,* and the N_2O reductase gene *nosZ* as molecular markers for denitrifier bacteria. The detected numbers of gene copies were lowest on day 8 for all four N-cycling-related genes (Fig. 2 b, c, e, f). Over the experimental period, the abundance of archaeal *amoA*, *nirK and nosZ* showed an increasing trend in all the treatments (Fig. 2 b, e, f), with a significant increase observed in the abundance of archaeal *amoA* from day 16 to day 56 (Fig. 2 b). While the abundance of bacterial *amoA* (Fig. 2 c) increased

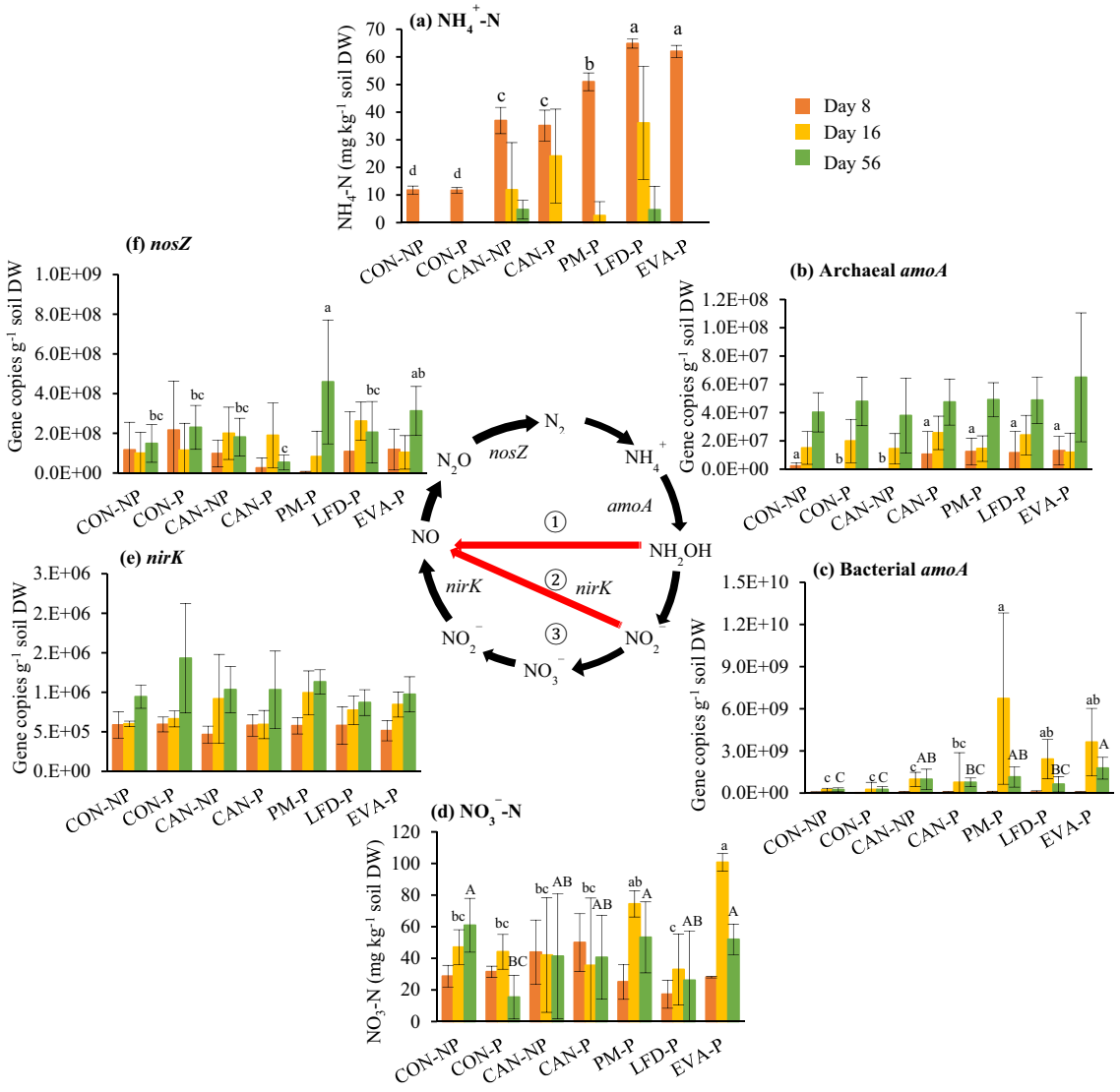

Fig. 2. The N cycle involving the nitrifier nitrification (pathway ①), the nitrifier denitrification (pathway ②) and the classical denitrification (pathway ③). The subfigures present the dynamics of soil NH_4^+-N (a) and NO_3^--N (d) concentrations (mg kg^{-1} soil DW, mean ± standard deviation, $n = 4$) and the abundance of N-cycling related genes (copies g^{-1} soil DW, mean ± standard deviation, n = 4): Archaeal *amoA* (b), Bacterial *amoA* (c), *nirK* (e) and *nosZ* (f) on day 8, 16 and 56. The lowercase and uppercase letters refer to statistical differences ($p < 0.05$) between treatments detected on day 8, day 16 or day 56, respectively. CON-NP, unplanted control with no N fertilizer applied; CON-P, planted control with no N fertilizer applied; CAN-NP, unplanted synthetic reference using calcium ammonium nitrate (CAN) as N fertilizer; CAN-P, planted synthetic reference using CAN as N fertilizer; PM-P, planted biobased treatment using pig manure; LFD-P, planted biobased treatment using liquid fraction of digestate; EVA-P, planted biobased treatment using evaporator concentrate.

in all the treatments from day 8 to day 16, and decreased to a lower level (1–2 × 10^9 gene copies g^{-1} soil DW) in PM-P, LFD-P and EVA-P by day 56. Among the treatments, on day 8, significantly higher gene copies of archaeal *amoA* were found in CON-NP, CAN-P, PM-P, LFD-P and EVA-P as compared to those in CON-P and CAN-NP (Fig. 2 b). The PM-P and EVA-P also resulted in a significantly higher abundance of bacterial *amoA* on day 16 and day 56 as compared to that in CON-NP and CON-P (Fig. 2 c), as well as significantly higher numbers of *nosZ* gene copies compared to CAN-P (Fig. 2 f). However, no significant difference was observed in the abundance of *nirK* between different treatments (Fig. 2 e).

The soil pH-H_2O values on day 8 decreased by 0.23 on average in CON-NP, CON-P, CAN-NP, CAN-P and EVA-P but increased by 0.10 on average in PM-P and LFD-P, as compared to the initial value (pH-H_2O = 7.26, Table 2). With the progress in N mineralization and maize growing, the soil pH-H_2O firstly decreased and then increased to approximately the initial level by day 56, except in EVA-P (6.92 ± 0.03) which was significantly lower than others (Table 4). The $EC_{1:5}$ values in biobased treatments (PM-P, LFD-P and EVA-P) showed an opposite trend, with an average increase of 30 μS cm^{-1} from day 8 to day 16 and an average decrease of 37 μS cm^{-1} from day 16 to day 56. Over the experimental period, the soil TN declined in all treatments except CON-NP. By the end of the experiment, the soil TN declined 6–11 % as compared to the initial value (979 ± 33 mg N kg^{-1} soil DW) on day 0.

Vegetation resulted in significantly higher soil pH-H_2O but significantly lower WFPS, soil $EC_{1:5}$, SMN and extractable K in CON-P as compared to CON-NP by day 56 (Table 4). Whereas in the application of CAN, vegetation only resulted in lower extractable P and K in CAN-P as compared to CAN-NP. When comparing the planted treatments, the application of N fertilizers, either synthetic or biobased, resulted in significantly higher $EC_{1:5}$ as compared to CON-P, with the highest values (276–348 μS cm^{-1}) observed in EVA-P throughout the experimental period. By day 56, the fertilized treatments also showed higher values of SMN, MBC, and a higher decline of TN (except PM-P) than those in CON-P, while all the biobased treatments showed higher extractable Na in soil than those of CON-P and CAN-P.

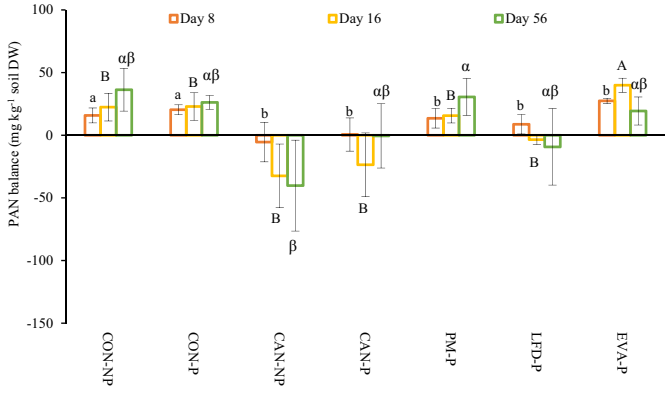

Fig. 3. The plant-available N balance calculated from input and output within the plant-soil system. A positive balance indicates net N mineralization, while a negative balance indicates net N immobilization or N loss. The lowercase and uppercase Alphabet letters and lowercase Greek letters refer to statistical differences ($p < 0.05$) between treatments detected on day 8, day 16 or day 56, respectively. CON-NP, unplanted control with no N fertilizer applied; CON-P, planted control with no N fertilizer applied; CAN-NP, unplanted synthetic reference using calcium ammonium nitrate (CAN) as N fertilizer; CAN-P, planted synthetic reference using CAN as N fertilizer; PM-P, planted biobased treatment using pig manure; LFD-P, planted biobased treatment using liquid fraction of digestate; EVA-P, planted biobased treatment using evaporator concentrate.

3.3. The N balance within the plant-soil system

The calculated balance of PAN, i.e., a sum of SMN and plant N uptake, was comparable in CON-NP and CON-P throughout the experimental period. The calculated PAN balance (Fig. 3) was always positive in CON-NP, CON-P, PM-P, and EVA-P, indicating net N mineralization. In the case of CON-P on day 16, PM-P and LFD-P on day 8, and EVA-P on day 16 and day 56, the PAN balance was calculated positive, suggesting a significant N mineralization which overweighed the PAN loss. While in CAN-NP, the PAN balance remained negative over the three sampling moments, indicating net N immobilization or N loss from the plant-soil system. For CAN-P and LFD-P, net N mineralization (positive values of PAN balance) was observed on day 8, and then net N immobilization or N loss (negative values of PAN balance) happened on day 16 and day 56; however, the absolute values were not significantly different from zero (equilibrium). Comparing between treatments, EVA-P showed the highest net N mineralization on day 8 (27.4 ± 2.1 mg N kg^{-1} soil DW) and day 16 (39.9 ± 5.6 mg N kg^{-1} soil DW) while CON-NP resulted in the highest net N mineralization on day 56 (36.3 ± 17.0 mg N kg^{-1} soil DW). The net N immobilization or N loss was highest in CAN-NP at all three sampling moments, with mean values of 5.5, 32.4, and 40.2 mg N kg^{-1} soil DW on day 8, day 16, and day 56, respectively.

3.4. Pearson correlation and principal component analysis for planted treatments

On day 56 in the planted treatments, SMN showed positive correlations with soil $EC_{1:5}$ ($r = 0.634$, $p < 0.01$), MBC ($r = 0.562$, $p < 0.01$), and the N concentration in maize shoot ($r = 0.456$, $p < 0.05$) and root ($r = 0.454$, $p < 0.05$). However, no significant ($p = 0.389$) correlation was found between SMN and N uptake in the whole plant. The soil $EC_{1:5}$ revealed significant and positive correlations with the soil extractable Na ($r = 0.718$, $p < 0.01$), MBN ($r = 0.530$, $p < 0.05$) and the abundance of bacterial *amoA* ($r = 0.585$, $p < 0.01$), as well as the Na in root ($r = 0.811$, $p < 0.01$). While the root Na showed a significant and negative impact on the plant shoot biomass ($r = -0.569$, $p < 0.01$) and the plant uptake of N ($r = -0.603$, $p < 0.01$), K ($r = -0.709$, $p < 0.01$), P ($r = -0.700$, $p < 0.01$) as well as S ($r = -0.586$, $p < 0.01$). No significant ($p > 0.05$) correlation was observed between the abundance of archaeal *amoA*, *nirK* and *nosZ* with other detected variables within this plant-soil system.

Fig. 4 depicts the overall grouping of individual observations of soil properties on day 56 and variable correlations extracted from the PCA results. The first two factors (PC1 and PC2) explained 55.2 % of the total variance in all the presented variables. Separation along PC1, which accounts for 39.8 % of the total variation, was explained mainly by differences in soil pH-H_2O, $EC_{1:5}$, SMN, extractable Na and abundance of bacterial *amoA* gene. The second factor (PC2) which accounts for 15.4 % of the total variation, was described mainly by differences in WFPS and abundance of archaeal *amoA* and *nirK* genes.

Based on the loadings on PC1 and PC2, the five planted treatments were separated into two groups (Fig. 4 b): the CON-P, CAN-P, PM-P, and LFD-P were grouped in the left part of the diagram, while EVA-P was separated towards the right part of the diagram regarding a significantly lower pH-H_2O but a higher $EC_{1:5}$ as well as the higher values in SMN, soil extractable Na, and the abundance of bacterial *amoA*.

4. Discussion

4.1. The N cycle and associated genes within the plant-soil system

The N cycle in soil is mainly driven by plant uptake and microbial mineralization-immobilization turnover (MIT) process (Meier et al., 2017). At the first sampling (day 8) of this study, net N mineralization occurred in all the treatments except CAN-NP (Fig. 3), due to the rewetting of air-dried soil on day 0 which activated the soil microbial

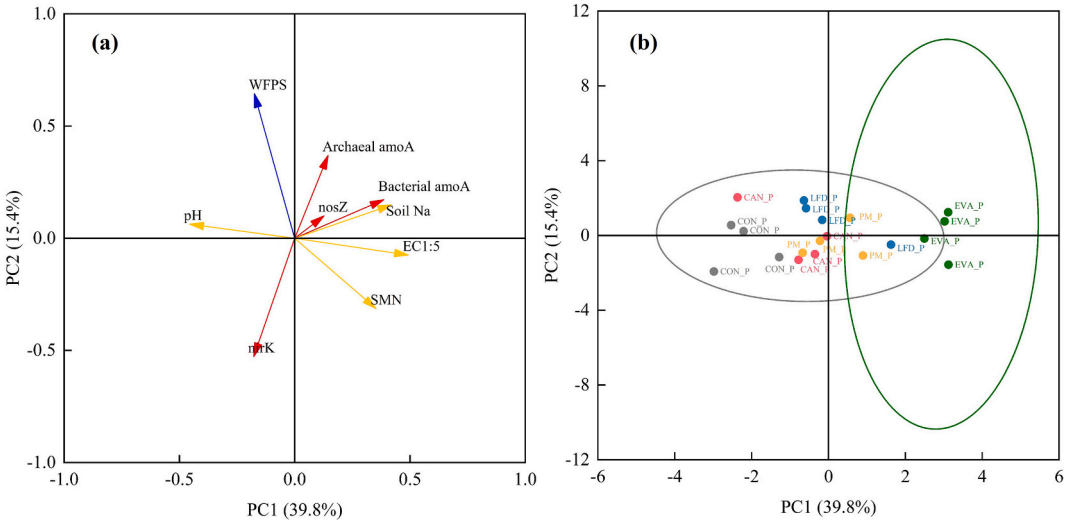

Fig. 4. Principal component analysis (PCA) based on the measurements of soil physical and chemical properties and the abundance of N-cycling-related genes on day 56. (a) PCA output. PC1 explains 39.8 % of the variance in the data and PC2 explains 15.4 % of the variance in the data. Solid lines with an arrow show the projections of the initial variables in the factorial space: blue lines indicate soil physical property (i.e. WFPS), yellow lines indicate soil chemical characteristics (i.e. pH-H_2O, $EC_{1:5}$, SMN and soil Na), red lines indicate soil biological properties (i.e. the abundance of bacterial and archaeal *amoA* and *nirK*, *nosZ* genes). (b) Position of the treatments in the factorial space. The gray circle represents the confidence interval of the group consisting of CON-P, CAN-P, PM-P and LFD-P while the green circle represents the confidence interval of EVA-P. WFPS, water-filled pore space; SMN, soil mineral nitrogen; CON-P, planted control with no N fertilizer applied; CAN-P, planted synthetic reference using calcium ammonium nitrate (CAN) as N fertilizer; PM-P, planted biobased treatment using pig manure; LFD-P, planted biobased treatment using liquid fraction of digestate; EVA-P, planted biobased treatment using evaporator concentrate.

activities and promoted soil organic matter (SOM) decomposition (Bapiri et al., 2010). Preplant fertilization also led to increases in PAN (Fig. 3), with CAN providing N in the form of NH_4^+-N and NO_3^--N at a ratio of 1:1, whereas biobased fertilizers (i.e., PM, LFD, and EVA) provided mainly NH_4^+-N (Table 1). The NH_4^+ in the soil is quickly oxidized into NO_2^- by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and further converted into NO_3^- by nitrite-oxidizing bacteria (NOB). In the microcosm incubations of Orellana et al. (2019) using isotopically labeled ammonium and urea, the concentration of NH_4^+ started to decline from day 2 while significant accumulation of NO_3^- started only from day 5 after application. In this study, the nitrification process seemed to become significant only after day 8 following fertilization, as indicated by the higher NH_4^+-N concentrations (Fig. 2 a) but comparable NO_3^--N concentrations (Fig. 2 d) in the fertilized treatments (CAN-NP, CAN-P, PM-P, LFD-P and EVA-P) as compared to the unfertilized treatments (CON-NP, CON-P) at the first sampling (i.e. day 8). Meanwhile, the detected gene copies of bacterial and archaeal *amoA* were relatively low on day 8 as compared to day 16 and day 56 (Fig. 2 b, c), suggesting a lower potential nitrification rate (Ren et al., 2020; Wang et al., 2014) on day 8. It was reported that compared to AOA, AOB is often predominant in soil with greater inorganic N availability (Di et al., 2009; Meyer et al., 2013; Strauss et al., 2014; Verhamme et al., 2011). This could explain the higher gene abundance of bacterial *amoA* (Fig. 2 c) compared to archaeal *amoA* in this study (Fig. 2 b), suggesting a stronger nitrification capacity associated with AOB than with AOA (Huang et al., 2021; Meinhardt et al., 2018; Wang et al., 2014). According to the field observation of Ouyang et al. (2017), the net and gross nitrification rates were mediated by AOB in the first weeks following fertilization; then, after NH_4^+ was depleted, the activity was dominated by AOA. The shift of dominant ammonia-oxidizing microorganisms between AOA and AOB indicated that AOA might be more likely affected by the oxidation of organic N compounds, as suggested already by others (Laanbroek et al., 2018; Levicnik-Hofferle et al., 2012). The increasing pattern of archaeal *amoA* gene abundance in this study (Fig. 2 b) also suggested an increasing dominance of AOA following the consumption of NH_4^+. However, by the end of the experiment (day 56), when concentrations of NH_4^+-N declined to a level lower than 5 mg N kg^{-1} soil DW, the number of archaeal *amoA* gene copies was still 5–10 fold lower than the number of bacterial *amoA* gene copies. This might be attributed, in part, to the constant temperature (16–21 °C) and moisture (60 % WFPS) conditions maintained in this pot experiment which favored bacteria over archaea (Guo et al., 2022).

Though a decline of NH_4^+-N concentration was observed in all the treatments (Fig. 2 a) from day 8 to day 56, in fertilized treatments it was

Table 3
Biomass (g $plant^{-1}$, mean ± standard deviation, $n = 4$) of maize shoot and root in dry weight (DW) and nutrient (N, P, K, S, Na) uptake (mg $plant^{-1}$, mean ± standard deviation, n = 4) in whole maize plant by day 56.

Parameter	unit	CON-P†	CAN-P	PM-P	LFD-P	EVA-P
Shoot DW	g $plant^{-1}$	3.8 ± 0.3 a‡	3.8 ± 0.6 a	3.6 ± 1.0 a	3.2 ± 0.3 ab	2.7 ± 0.2 b
Root DW	g $plant^{-1}$	2.4 ± 0.5 a	3.0 ± 1.0 a	2.9 ± 0.2 a	2.1 ± 0.5 a	2.4 ± 0.1 a
N uptake	mg $plant^{-1}$	140 ± 10 a	143 ± 25 a	141 ± 33 a	114 ± 25 ab	103 ± 5 b
P uptake	mg $plant^{-1}$	13 ± 1 a	12 ± 2 ab	12 ± 3 ab	10 ± 2 bc	9 ± 1 c
K uptake	mg $plant^{-1}$	246 ± 32 a	233 ± 43 a	219 ± 39 a	203 ± 31 ab	157 ± 16 b
S uptake	mg $plant^{-1}$	15 ± 2 a	13 ± 2 ab	12 ± 1 bc	12 ± 1 bc	11 ± 1 c
Na uptake	mg $plant^{-1}$	2.01 ± 0.33 a	1.87 ± 0.51 a	1.98 ± 0.43 a	2.53 ± 0.49 a	2.74 ± 0.43 a

† CON-P, planted control with no N fertilizer applied, CAN-P, planted synthetic reference using calcium ammonium nitrate (CAN) as N fertilizer, PM-P, planted biobased treatment using pig manure, LFD-P, planted biobased treatment using liquid fraction of digestate, EVA-P, planted biobased treatment using evaporator concentrate.

‡ Values with the same lowercase letters in a row within the Sub-spanner heading are not significantly different at $P < 0.05$.

not fully offset by the plant N uptake (Table 3) and the increase of soil NO_3^--N (Fig. 2 d). Assuming the PAN balance in CON-P and CON-NP represented the soil N supply under planted and unplanted conditions, respectively, the negative PAN balance in the CAN-NP, CAN-P and LFD-P (Fig. 3) could be attributed to inhibited SOM decomposition upon fertilization (He et al., 2016), stimulated N immobilization (Wei et al., 2020a) or N losses through leaching, volatilization or emissions in the form of NO, N_2O or N_2 gas (Fangueiro et al., 2018; Wei et al., 2020b). In this study, the N leaching was reduced to a negligible level by returning all the leachate to the soil, and the NH_3 volatilization loss upon soil incorporation was assumed to be <10 % of applied NH_4^+-N, i.e., < 6 mg N kg^{-1} soil DW according to the application rate (31–49 mg NH_4^+-N kg^{-1} soil DW) in this study (Huijsmans and Hol, 2011; Klop et al., 2012). Consequently, gaseous N loss as NO, N_2O or N_2 seemed to be the predominant reason for a negative PAN balance (Fig. 3).

Gaseous N loss could happen via three possible pathways: (i) nitrifier nitrification which involves the transformation of hydroxylamine (NH_2OH) to NO and N_2O (pathway ① in Fig. 2); (ii) nitrifier denitrification which involves the reduction of NO_2^- to NO and N_2O before oxidation to NO_3^- (pathway ② in Fig. 2); and (iii) the classical denitrification process involving the reduction of NO_3^- to NO_2^- and NO, which are further converted into N_2O or N_2 (pathway ③ in Fig. 2). The biotic transformation of NH_2OH into NO is a newly proposed pathway (Caranto and Lancaster, 2017) that has only been shown in-vitro; thus, the possibility of occurrence in conditions of this experiment will need further investigation and confirmation. However there is also a possibility of abiotic degradation of NH_2OH into NO and N_2O (Duan et al., 2020) which could happen sooner than the biotic pathway and dominate the gaseous N loss in short term after fertilization (Liu et al., 2019). In addition, heavy NH_4^+ application from CAN and biobased fertilizers may have boosted the ammonia-oxidizer activities resulting in a pH decrease (Section 3.2) and NO_2^- accumulation, given a relatively high nitrification rate but a low O_2 availability which repressed the synthesis of nitrite reductase (Van Cleemput and Samater, 1995). Unfortunately, the concentration of NO_2^- was not determined in this study so we cannot confirm the assumption. However, it has been reported that the accumulation of NO_2^- in the soil acts as an important driver of NO_x and N_2O production by both biotic and abiotic reactions (Heil et al., 2016). Given the WFPS was maintained at around 60 %, which was not conducive to a predominant production of N_2O through denitrification (Bateman and Baggs, 2005), there could be a higher possibility of nitrification and nitrifier denitrification as a main contribution to the gaseous N losses from this experiment (Kool et al., 2011). Regardless, the application of nitrification inhibitors that selectively inhibit the activities of AOB, i.e., the ammonia oxidation process (Papadopoulou et al., 2020), should be considered a good strategy to reduce the N losses through nitrifier nitrification, nitrifier denitrification or nitrate leaching (Guo et al., 2022), especially when ammonium-rich fertilizers, like the biobased fertilizers of this study, have to be applied at preplant.

Regardless of the significant correlations found in other studies between the abundance of *nirK* gene and soil pH (Li et al., 2021; Xiao et al., 2021) as well as soil moisture (Xu et al., 2020), the abundance of *nirK* gene detected in this study revealed no significant correlation with other variables (Fig. 4 a) neither significant difference among treatments (Fig. 2 e). In a laboratory incubation experiment, Cui et al. (2016) observed higher N_2O emissions as well as a higher abundance of *nirK*, *nirS* and *nosZ* in a soil that received pig manure for 30 years than an unfertilized control and an inorganically fertilized treatment. In contrast, Tatti et al. (2013) reported no significant correlation between the abundance of the denitrification genes (*nirK*, *nirS* or *nosZ*) and the N_2O emissions or denitrification rates. In this experiment, PM-P and EVA-P also resulted in higher gene copies of nosZ by day 56 (Fig. 2 f), however, since we did not measure the fluxes of N_2, NO or N_2O, it is difficult to link the abundance of nirK or nosZ genes with the gaseous N losses.

4.2. Vegetation reduced the N losses upon preplant fertilization

A key factor that might impact the NUE of fertilizers applied at preplant is the interaction between plant root growth and soil microbial activities, which affects N cycling (Grunert et al., 2019). In this study, the effect of vegetation effect on the soil's chemical and biological properties was investigated through the set-up of a planted and unplanted treatment with no-N applied (CON-P and CON-NP) or using CAN as N fertilizer (CAN-P and CAN-NP), respectively. Within the first two weeks after sowing, the maize plants were so small that the plant N uptake was only 3–10 % of the PAN in the planted treatments. Therefore, by day 8, net N immobilization or N loss could have occurred already in both CAN-NP and CAN-P, as indicated by the negative PAN balance (Fig. 3). With the fast growth of maize shoots and roots from day 16 to day 56, vegetation started to significantly impact the soil properties (Fig. 2 and Table 4). By day 56, the PAN remained comparable in CON-P and CON-NP; however, it became higher in CAN-P than in CAN-NP. Consequently, though negative PAN balances (net N immobilization or N loss) were observed in both CAN-P and CAN-NP, the absolute value was lower in CAN-P (Fig. 3), suggesting that vegetation could have decreased the N immobilization or N loss upon preplant fertilization.

It was observed in the experiment of Jiang et al. (2021) that maize plants increased the MBC and MBN as compared to unplanted treatment, indicating an increased microbial N immobilization under vegetation.

Table 4
Soil chemical and biological properties (mean ± standard deviation, $n = 4$) by day 56.

Parameter[†]	unit	CON-NP[‡]	CON-P	CAN-NP	CAN-P	PM-P	LFD-P	EVA-P
pH-H_2O	/	7.17 ± 0.08 b[§]	7.31 ± 0.03 a	7.15 ± 0.08 b	7.19 ± 0.12 ab	7.30 ± 0.05 a	7.18 ± 0.09 b	6.92 ± 0.03 c
$EC_{1:5}$	μS cm^{-1}	234 ± 28 b	156 ± 20 c	229 ± 27 b	202 ± 39 b	200 ± 19 b	224 ± 29 b	286 ± 8 a
WFPS	%	69.4 ± 0.7 a	58.6 ± 1.7 bc	68.6 ± 1.2 a	65.4 ± 7.5 ab	54.6 ± 3.4 c	62.6 ± 6.0 abc	54.4 ± 4.6 c
TN	mg kg^{-1} DW	916 ± 29 a	860 ± 20 a	867 ± 22 a	897 ± 49 a	921 ± 28 a	897 ± 28 a	907 ± 39 a
SMN	mg kg^{-1} DW	61.2 ± 17.0 a	11.5 ± 6.5 b	46.1 ± 36.2 ab	43.3 ± 25.8 ab	53.6 ± 22.5 a	31.0 ± 27.6 ab	52.0 ± 9.7 a
MBC	mg kg^{-1} DW	116 ± 9 c	123 ± 4 c	126 ± 20 bc	147 ± 22 ab	151 ± 15 a	132 ± 13 abc	146 ± 16 ab
MBN	mg kg^{-1} DW	23.4 ± 13.0	15.6 ± 4.6	25.2 ± 19.9	21.8 ± 2.5	25.0 ± 10.2	16.0 ± 8.5	34.1 ± 17.7
Extractable P	mg kg^{-1} DW	241 ± 6 ab	240 ± 3 ab	243 ± 6 ab	232 ± 2 c	235 ± 4 bc	250 ± 16 a	240 ± 19 abc
Extractable K	mg kg^{-1} DW	210 ± 4 a	159 ± 6 c	217 ± 13 a	159 ± 5 c	175 ± 8 b	175 ± 9 b	170 ± 13 bc
Extractable S	mg kg^{-1} DW	103 ± 15 a	115 ± 24 a	119 ± 56 ab	86 ± 2 b	81 ± 7 b	96 ± 15 ab	128 ± 33 b
Extractable Na	mg kg^{-1} DW	26 ± 3 b	22 ± 4 b	23 ± 3 b	20 ± 2 b	40 ± 5 a	45 ± 4 a	55 ± 6 a

[†] pH-H_2O and $EC_{1:5}$ refer to values from water extract at a ratio of 1:5 w/v. Concentrations of extractable P, K, S and Na refer to the determination of nutrients from soil by ammonium lactate (pH = 3.75) extraction. WFPS, water-filled pore space; SMN, soil mineral N (i.e. NH_4^+-N + NO_3^--N); TN, total N; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

[‡] CON-NP, unplanted control with no N fertilizer applied; CON-P, planted control with no N fertilizer applied; CAN-NP, unplanted synthetic reference using calcium ammonium nitrate (CAN) as N fertilizer; CAN-P, planted synthetic reference using CAN as N fertilizer; PM-P, planted biobased treatment using pig manure; LFD-P, planted biobased treatment using liquid fraction of digestate; EVA-P, planted biobased treatment using evaporator concentrate.

[§] Lowercase letters following the values refer to statistical differences ($p < 0.05$) between treatments.

However, in this study, the MBC and MBN by day 56 revealed no significant difference between the planted and unplanted treatments (Table 4). There is also the possibility that the microbial immobilized N was rapidly transformed into the organic pool as microbial residues or necromass (Quan et al., 2016), which was thus not detectable in either MBN or SMN.

Fertilization was reported to significantly increase the denitrification in soil by 174 % as compared to unfertilized control (Wang et al., 2018), and this stimulation is even higher in the application of CAN (by 408 %) and other NO_3-based fertilizers (by 344 %). In this study, the application of CAN may have enriched the NO_3^- pool in the soil as a ready N source for denitrification, resulting in N loss as N_2O or N_2 (Tatti et al., 2013) during the first week after fertilization. Whereas under vegetation, the plant uptake of NO_3^--N as a major N sink in the plant-soil system may have promoted the N cycle towards the production of NO_3^- and thus reduced the gaseous N loss through nitrification and denitrification. Furthermore, based on the higher number of archaeal *amoA* gene copies detected in CON-P and CAN-P as compared to CON-NP and CAN-NP (Fig. 2 b), the presence of maize plant may have increased the potential nitrification rate associated with organic N mineralization (Laanbroek et al., 2018; Levicnik-Hofferle et al., 2012). This was in line with the pot experiment by Wang et al. (2015), who also observed increased soil nitrification potential (by 5 to 10-fold) in the planted treatment as compared to unplanted.

This experiment was limited to analyses during the first two months after fertilization only, and hence results should be interpreted with caution when evaluating the NUE of biobased fertilizers during the entire growing season. Regardless, the remaining SMN (average of 11.5 ± 6.5 mg N kg^{-1} soil DW) in CON-P by the end of the experiment (Table 4) suggested that the mineral N supplied by the native soil organic matter was more than enough to satisfy the N demand of the young maize plant. The surplus N from fertilizer application may have largely increased the risk of N losses from the plant-soil system, especially under preplant fertilization when no plant is presented (e.g., CAN-NP) or when plants were too young to rapidly take up appreciable amounts of the applied N (e.g., up to V6 growth stage of maize, accounting for one-third of its life cycle) (Ma et al., 2005). Therefore, in regions with high soil N-supplying capacity and thus with risks of excessive NO_3 accumulation, additional mitigation measures like cover crop cultivation during the fallow period will be needed to prevent further N losses through leaching or denitrification when biobased fertilizers are applied as the sole N source at preplant (Sanz-Cobena et al., 2014; Shelton et al., 2018).

4.3. Potential salt stress from biobased fertilizers to young plants

The full replacement of synthetic mineral N fertilizer, i.e., CAN, by PM and LFD revealed no significant impact on either plant growth (Table 3) or soil chemical and biological properties (Fig. 4 b). Despite a decline in TN (Section 3.2), the PAN balance in PM-P became increasingly positive from day 16 to day 56 (Fig. 3), indicating a higher rate of N mineralization than mineral N loss. It could be attributed to the rhizosphere priming effect, which promoted the decomposition of OM (both from soil and PM) by providing root exudates as a C source for soil microbes (Meier et al., 2017). Compared to LFD and EVA recovered after anaerobic digestion, the raw PM may contain a higher content of labile C (Möller, 2015) thus reflected a higher OM mineralization after application. However, it revealed the opposite trend in EVA-P, with an average 20 mg N kg^{-1} soil DW decrease in PAN from day 16 to day 56 (Fig. 3). Linking to the lower plant biomass (Table 3) but higher Na concentrations in the plant root (data not shown) and the soil (Table 4) of EVA-P, it could be assumed that the application of EVA may have brought excess Na to the plant-soil system and resulted in salt stress which weakens the competitiveness of plants against the microbes in N metabolism (Ma et al., 2020). The potential of salt stress was further supported by the positive correlations between soil $EC_{1:5}$ and soil extractable Na, whereas the negative correlations between soil extractable Na and the plant uptake of other nutrients (Section 3.4). Similar results were also observed in the pot experiment of Saju et al. (2022), wherein the application of evaporation concentrate exhibited poor yield and N uptake in lettuce compared to synthetic fertilizers. Salt stress affects various physiological and metabolic processes in plant growth by creating osmotic stress in the plant cell (Gupta and Huang, 2014). Maize is regarded as moderately sensitive to salt stress (Grieve et al., 2012); however, the response of plants varies with the degree of stress and crop growth stage (Farooq et al., 2015), e.g., germination and stand establishment are more sensitive to salt stress than later developmental stages. Though the toxic Na concentration (> 0.25 M as proposed by Menezes-Benavente et al., 2004) was not reached in this experiment, the young maize plant in EVA-P could have gone through slight salt stress, which resulted in suppression of the plant shoot and root growth as well as nutrients uptake (Table 3). However, this salt stress was not observed in the field application of EVA from the same nutrient recovery cascade (Luo et al., 2022), probably due to the lower application rate (105 kg N ha^{-1}) compared to this study (265 kg N ha^{-1}), or a significant impact of precipitation and leaching at field conditions (Eswar et al., 2021).

According to the observations of Guo et al. (2021), soil salinity ($EC_{1:5}$ > 500 μS cm^{-1}) led to decreased copy numbers of bacterial and archaeal *amoA* genes but increased those of denitrifier *nirS* and *nosZ* genes. Instead, Ma et al. (2019) reported a reduction in the abundance of *nirK*, *nirS,* and *nosZ* genes under salinity ($EC_{1:5}$ = 8040 μS cm^{-1}). While the study by Meng et al. (2020) indicated that soil salinity exerted a significant inhibitory impact on nitrification and denitrification, particularly when the EC of a saturated soil paste extract was >4050 μS cm^{-1}. However, the soil $EC_{1:5}$ of this study was relatively low (< 300 μS cm^{-1}) compared to the abovementioned studies, which might explain the limited impact on the abundance of archaeal *amoA*, *nirK* and *nosZ*. Moreover, cautions should be taken when linking results obtained under controlled conditions during the short-term period to those in the field-scale and long-term application, considering the significant impact of environmental factors like weather conditions on crop growth (Hernández et al., 2013) and soil microbial activities (Urakawa et al., 2017).

5. Conclusions

Results of this pot experiment highlighted the potential of manure-derived biobased fertilizers in fully substituting synthetic mineral fertilizer (i.e. calcium ammonium nitrate) at preplant fertilization. Compared to bare soil, the presence of maize plants reduced N losses under synthetic mineral N fertilization, probably benefiting from the continuous plant N uptake and stimulated organic N mineralization, as indicated by the higher number of archaeal *amoA* gene copies observed under planted treatments. Application of pig manure and liquid fraction of digestate further reduced the N losses as compared to the synthetic reference, without significant impact on the plant growth and soil microbial communities. However, applying concentrated biobased fertilizers derived from manure (i.e., evaporator concentrate in this study) as the sole N source may pose a risk of sodium excess, potentially resulting in salt stress to young plants and causing a reduction in biomass yield and nutrient uptake. The long-term performance of these products should also be further evaluated at field-scale to realistically reflect the N fertilizer value during consecutive seasons.

CRediT authorship contribution statement

Hongzhen Luo: Conceptualization-experimental design, Methodology, Formal analysis, Investigation, Validation, Writing-original draft, Writing-review & editing. **Massimo Zilio**: Methodology, Formal analysis, Investigation, Validation, Writing-review & editing. **Ivona Sigurnjak**: Conceptualization-experimental design, Methodology, Validation, Writing-review & editing. **Ana A. Robles-Aguilar**:

Conceptualization-experimental design, Methodology, Validation, Writing-review & editing. **Evi Michels**: Funding acquisition, Writing-review & editing. **Fabrizio Adani**: Methodology, Validation, Review & editing, Funding acquisition. **Stefaan De Neve**: Conceptualization-experimental design, Methodology, Supervision, Validation, Review & editing. **Erik Meers**: Conceptualization-experimental design, Methodology, Supervision, Validation, Review & editing, Funding acquisition. All authors contributed to manuscript reviewing & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.105033.

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