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REGULAR ARTICLE

Net-mineralization of organic matter and greenhouse gas emissions from Quebracho tannin-enriched manure applied to acidic and alkaline soils

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Abstract

Background: Mitigation strategies to reduce greenhouse gas emissions, ammonia volatilization, and nitrate leaching from agriculture are important for the management of environmental pollution and climate change. Tannins, which are water-soluble polyphenolic compounds, were found to reduce emissions in animal husbandry systems when supplemented to ruminant diets.

Aim: Two laboratory incubation experiments were conducted to investigate the effects of Quebracho tannin-enriched manure on carbon (C) and nitrogen (N) mineralization processes, and greenhouse gas emissions from three different soils (sandy moderate acidic, sandy alkaline, and loamy alkaline).

Methods: In the two incubation experiments of 4 and 9 weeks, soil samples were analyzed for K_2SO_4 extractable C and N, NH_4^+ and NO_3^-, microbial biomass C, and pH. In the main experiment emissions of CO_2, N_2O, and NH_3 were regularly monitored.

Results: Three days after manure application, the CO_2 emissions of the three soils were reduced by 26%–37% and N_2O by 80%–92% in tannin-enriched manure treatments compared with tannin-free manure. However, subsequent cumulative CO_2 and N_2O emissions were only reduced in the loamy alkaline soil applied with tannin-enriched manure. Also, in the initial 4 weeks net-mineralization after manure application was significantly lower in soils applied with tannin-enriched manure, reflecting an immobilization of N compared with tannin-free manure in the short term or an inhibition of the mineralization process by physicochemical complexation of proteins or inhibition of enzyme activities in the long term. In both experiments, NO_3^- was by 23%–95% lower in the three soils after 3–9 weeks compared with tannin-free manure.

Conclusion: These results highlight the mitigation potential of tannin-enriched manure on soil N leaching, and suggest that greenhouse gas emissions may be increased depending on soil properties.

KEYWORDS

CO_2, condensed tannins, N_2O, NH_3 emission, tropical sandy soil, turnover processes

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1 | INTRODUCTION

Agricultural soils, particularly under intensive cultivation practices, are major contributors to greenhouse gas emissions (Mu et al., 2013). Further, 57% of the global ammonia (NH_3) emissions are caused by the agricultural sector (Sutton et al., 2013). Ammonia volatilization can account for up to 65% of the fertilizer N input and are particularly large on sandy calcareous soils, with low soil organic C content and low cation exchange capacity (Martens and Bremner, 1989). Mineralization processes and thus gaseous C and N losses are increased due to the favorable conditions for microbial decomposition under the hot and moist conditions of irrigated tropic and subtropic soils (Siegfried et al., 2011; Ingold et al., 2017). In intensively cultivated irrigated vegetable fields and urban gardens of Oman and Niger, at least 6%–11% of the N applied in the form of animal manure at rates of 295 and 572 kg N ha^{-1} y^{-1} in total were lost via nitrous oxide (N_2O) and NH_3 (Predotova et al., 2010; Siegfried et al., 2011). In the latter studies gaseous CO_2-C losses reached 90%–135% of applied C at annual application rates between 8.8 to 13.7 Mg ha^{-1} y^{-1} of buffalo manure (Siegfried et al., 2011) and total CO_2-C losses of up to 20–26 Mg ha^{-1} y^{-1} were noted in strongly fertilized urban gardens in Niamey (Predotova et al., 2010). Besides the increase of greenhouse gas emissions, the increase of organic matter mineralization in intensively cultivated soils can lead to a depletion of soil organic matter and high nutrient leaching losses.

A potential mitigation strategy to reduce N_2O and NH_3 emissions from soil applied with animal manures is the supplementation of the diets of animals with tannin additives or tanniferous forages (Hristov et al., 2013). Reductions in N_2O emission and NH_3 volatilization from animal manure and slurry are accomplished by redirecting N excretion from labile N in urine to more stable forms in feces (Hess et al., 2006; Misselbrook et al., 2005; Powell et al., 2011a; Waghorn, 2008). This redirection is due to the antimicrobial property of tannins, its proteins binding capacity, and its enzymes inhibiting potential (Adamczyk et al., 2017). These attributes may protect fodder proteins and carbohydrates from microbial degradation within the animals' digestive tract, which can lead to a reduction in feed digestibility and an alteration in protein utilization by animals with detrimental effects on animal health (Al-Kindi et al., 2016; Mueller-Harvey, 2006; Waghorn, 2008). Despite this, numerous studies reveal that depending on tannin type and quantity, tannins can have beneficial effects on the animals' protein use efficiency, health status (reduction of bloat and parasite infections), and milk, meat, and wool productivity (Mueller-Harvey, 2006; Waghorn, 2008). In addition, tannin-containing browse plant species can be important feed sources particularly for goats in traditional animal husbandry (Schlecht et al., 2011), and their presence in many fodder plants, is crucial in many (agro-) ecosystems (Aerts et al., 1999).

During the passage of feed through an animal's digestive tract, some polyphenols, such as hydrolysable tannins, are degraded or metabolized by rumen microbes (Makkar, 2003a). However, condensed tannins, which are a group of relatively stable compounds with high protein binding affinity, cannot be degraded by rumen microbes and are possibly excreted with feces (Makkar et al., 1995). Therefore, manure from animals fed on condensed tannin-containing fodder, such as *Lotus corniculatus* L., contain tannins and additionally have increased total N contents in more stable forms as shown by Powell et al. (2009). This altered manure quality can affect manure decomposition and mineralization after soil application. Most studies reporting tannin effects on decomposition processes deal with polyphenols from plant litter, which are to a large extent soluble and thus easily react with proteinaceous material in soils (Kraus et al., 2004). Soluble tannins are more reactive, they inhibit soil enzyme activity and some microorganism traits, however, they are also more quickly degraded and inactivated, whereas insoluble tannins bound to organic compounds are more stable and likely have long-term effects on mineralization processes.

In a field experiment in Northern Oman, a condensed tannin extract from Quebracho (*Schinopsis balansae*), fed to goats as a feed additive, decreased decomposition of soil applied manure compared to pure goat manure or a mix of goat manure with the same water-soluble tannin extract (Ingold et al., 2017). Furthermore, in the same study, ingested Quebracho tannins strongly reduced relative N release from manure, whereas water soluble tannins added with goat manure to the soil had less pronounced effects. This reduction in N release resulted in lower N uptake by cultivated maize (*Zea mays* L.) and radish (*Raphanus sativus* L.) (Ingold et al., 2015) indicating an inhibition of N mineralization processes, which may result in lower N-losses (*e.g.*, greenhouse gas emissions) from soil-applied manures.

The potential of tannin enriched manures on the mitigation of greenhouse gas emissions calls for further research to investigate the effect on organic matter decomposition, nutrient cycling, and greenhouse gas emissions in manured soils (Hristov et al. 2013). In this study we hypothesized that application of manure from goats which ingested Quebracho tannins leads to (1) a decreased net-mineralization of organic N from manure in soils, (2) lower NH_3 volatilization, and CO_2 and N_2O emissions, and (3) that removal of water-soluble tannins by washing has minor effects on net-N-immobilization. To test these hypotheses, two incubation experiments were carried out. A 4-week preliminary experiment was conducted to investigate the effect of Quebracho tannins in goats' diets on mineralization processes of soil applied manure in two contrasting sandy subtropical topsoils. Hereby, the contribution of water-soluble tannins to the overall effect of tannin was tested. The main experiment was conducted with the same two sandy subtropical topsoils and a contrasting loamy alkaline temperate subsoil, and similar manure application rates to further corroborate the findings using additional frequent greenhouse gas measurements during a 9-week incubation period.

2 | MATERIALS AND METHODS

2.1 | Soils

Incubation experiments were carried out using two subtropical sandy topsoils with pH_{H2O} values of 5.5 and 8.8 and a contrasting temperate alkaline subsoil (Table 1). The sandy alkaline soil was classified as a hyperthermic Typic Torrifluvent (US Taxonomy; Al-Farsi & Cookson, 2002) derived from Wadi deposits and collected near Sohar

TABLE 1 Properties of soils used in the incubation experiments with standard deviations in parentheses

Soil properties		Sandy acidic topsoil		Sandy alkaline topsoil		Loamy alkaline subsoil	
Sand	(%)	94.0	(0.05)	84.4	(1.23)	18.5	(0.15)
Silt	(%)	2.6	(0.24)	12.2	(0.88)	65.9	(0.73)
Clay	(%)	3.4	(0.19)	3.5	(0.34)	15.6	(0.58)
WHC	(%)	25.4	(0.16)	30.1	(1.13)	31.3	(0.61)
pH_{H2O}		5.7	(0.08)	8.8	(0.03)	8.3	(0.04)
Carbonate	(%)	0.1	(0.00)	5.0	(0.25)	9.7	(0.78)
SOC	($mg\ g^{-1}$)	1.8	(0.13)	6.1	(0.25)	3.1	(0.01)
extr.C	($\mu g\ g^{-1}$)	30.8	(2.85)	79.6	(6.66)	24.4	(5.07)
Total N	($\mu g\ g^{-1}$)	220.0	(16.70)	396.5	(7.10)	128.5	(12.46)
extr. N	($\mu g\ g^{-1}$)	35.1	(3.76)	24.3	(0.82)	3.9	(0.76)
NH_4	($\mu g\ g^{-1}$)	11.7	(0.59)	0.3	(0.20)	1.4	(0.26)
NO_3	($\mu g\ g^{-1}$)	29.6	(0.86)	20.3	(1.08)	1.2	(0.42)

Abbreviations: WHC = Water holding capacity, SOC = Soil organic matter

TABLE 2 Properties of goat manure used in the incubation experiments

Manure properties		Tannin-free manure (M)	Tannin-manure (T)	Tannin-manure washed (Tw)
Ash	(%)	8.0	7.2	8.0
OM	(%)	92.0	92.8	92.0
C	(%)	46.0	46.1	46.2
N	(%)	2.7	2.4	2.4
C N ratio		17.2	19.4	19.3
P	($mg\ g^{-1}$)	8.3	6.7	6.8
K	($mg\ g^{-1}$)	2.3	2.1	1.3
Na	($mg\ g^{-1}$)	2.3	2.7	3.7
Total phenols	(mg TA eq. g^{-1})	1.09	3.09	2.61
Total tannins	(mg TA eq. g^{-1})	0.65	2.63	2.24
Cond. tannins	(mg LC eq. g^{-1})	0.06	0.69	0.65

Abbreviations: OM = Organic matter, TA eq. = Tannic acid equivalent, LC eq. Leucocyanidin equivalent

(24°22'N, 56°34'E, 15 m asl), in the Al-Batinah plain of the Sultanate of Oman from cultivated farmland (Ingold et al., 2015). The sandy and moderately acidic soil was a Psammentic Paleustalf collected in Sadoré, Niger (13°14'N, 2°17'E, 235 m asl). The contrasting subsoil (pH_{H2O} 8.3) was derived from Loess, sampled at 3 m depth of a break-off edge of a dry valley under spruce-beech mixed forest vegetation near Witzenhausen (51°21'N, 9°51'E, 4 m asl; Mamo et al., 1996). It was classified as a C horizon of a cambisol. Prior to the incubation experiments the three soils were air-dried and stored at room temperature.

2.2 | Manure production and analysis

Tannin-free goat manure (M) and Qurebracho tannin-enriched manure (T) were collected in a digestibility trial using 48 male Jabal Akhdar goats of similar age (Ingold et al., 2015). The goats received a basal diet of 50% Rhodes grass hay (*Chloris gayana* Kunth.), 47% maize, and 3% soybean cake [*Glycine max* (L.) MERR.], T was produced with 3.4% Quebracho extract in the animals' daily ration. After a 14-day adaptation period, manure was collected in specially designed fabric bags attached to the goats back once a day. The collected manure was sun-dried until constant weight, a common procedure in Oman, and stored at room temperature under dry conditions. The manure was then crushed and sieved to 2 mm to obtain homogeneous subsamples and analyzed for nutrient and tannin concentrations (Table 2). A subsample of the tannin-enriched manure was washed with deionized water to remove soluble tannins (Tw) and dried thereafter. Total C and N in manure remained unchanged by this procedure. The manure was analyzed for dry matter after drying at 105°C, for ash content by ashing the sample at 550°C for 16 h, and for organic matter (OM) calculated as the difference between dry matter and ash content. Total P, K, and Na were extracted from the ash in 20 mL 32% HCl for 24 h and diluted with H_2O to 100 mL before filtration. Phosphorus was measured colorimetrically (VDLUFA, 1976) by a spectrophotometer (U-2000, Hitachi Ltd., Tokyo, Japan). Potassium and Na were measured by flame photometry (Flame photometer, BWB Technologies Ltd, Newbury, UK). Total C and N were determined in powder milled dry material using a VarioMax® CHN analyzer (Elementar Analysesysteme GmbH, Langenselbold, Germany). Total phenols were measured in 70% acetone-water extracts (400 mg dried and milled sample with 20 mL acetone-water) by the Folin-Cioucalteau method using a Folin-Ciocalteu's phenol reagent (Sigma-Aldrich Chemie GmbH, Munich, Germany; Makkar, 2003b) and 20% Na_2CO_3. After 30 min of reacting time in the dark, phenol concentrations were determined as tannic acid equivalents with a photometer (Specord 50 Plus, Analytik Jena AG, Germany) at 725 nm. Tannins were calculated as the difference between total phenols and non-tannin phenols, which were analyzed by the Folin-Ciocalteu method after precipitating tannins using polyvinylpolypyrrilidone (PVPP; Sigma-Aldrich Chemie GmbH, Munich, Germany). To this end, 100 mg of PVPP were mixed with 800 µL H_2O and 800 µL extract, cooled for 15 min, mixed and cooled again for 15 min, and centrifuged at 3800 rpm for 5 min. The supernatant was used to analyze the non-tannin phenols. Condensed tannins were analyzed in the same extract by the butanol-HCl method described by Hagerman (2002) with 60 min reaction time at 96°C prior to colorimetric measurement at 550 nm.

2.3 | Incubation experiments

Prior to both incubation experiments, the soils were sieved to 2 mm, moistened with deionized water to 50% of the water holding capacity (WHC) and preincubated for 15 days at 22°C in plastic bags. After 11 days of pre-incubation, aliquots of 35 g soil dry matter were filled in four 68-mL polystyrol tubes and placed in an air-tight 1.6 L incubation container. After 4 days, evaporated soil water was replenished and the main incubation started with the application of manure.

In the preliminary experiment, crushed goat manure (M, T, or Tw) was mixed into the two subtropical topsoils at a rate of 2.9 g kg^{-1} soil (equivalent to about 150 kg N ha^{-1}), thereafter the soils were recompacted to a bulk density of about 1.45 g cm^{-3} and incubated for 30 days. Soil samples were taken at the beginning and after 30 days for laboratory analysis.

In the main experiment, the two subtropical sandy topsoils and a temperate loamy subsoil were incubated with manure (M and T) at a rate of 2.9 g kg^{-1} soil under similar conditions as in the preliminary experiment for a duration of 63 days. Un-amended soil served as a zero control (C). Gas emissions for CO_2 and N_2O were monitored 0, 1, 2, 3, 6, 9, 13, 20, 30, and 53 days after manure application, NH_3 volatilization was sampled during initial two weeks, and soils samples taken on day 0, 21, and 63 days of incubation for laboratory analysis.

2.4 | Soil analysis

All soil samples were stored at 4°C until analysis. Microbial biomass C was estimated by fumigation extraction (Vance et al., 1987), whereby two aliquots (10 g) of each sample were either fumigated with chloroform or non-fumigated. The two aliquots were then extracted with 40 mL 0.5 M K_2SO_4, and total organic C and total N were analyzed in filtered extracts using an automatic analyzer multi-N/C® 2100 (Analytic Jena AG, Germany). The same non-fumigated extracts were analyzed for NH_4^+ and NO_3^- by a Continuous Flow Analyzer Evolution II (Alliance Instruments GmbH, Salzburg, Austria). Dried and milled soils were analyzed for total C and N by combustion using a VarioMax® CHN analyzer (Elementar Analysesysteme GmbH, Langenselbold, Germany) and soil pH was measured at a ratio of $1{:}2.5_{H_2O}$. Carbonate was analyzed gas-volumetrically using a Scheibler apparatus after the addition of 10% HCl (Schaller 2000) and carbonate-C was subtracted from total C concentrations to estimate soil organic C (SOC).

2.5 | Gas measurements

For gas analysis, incubation containers were flushed with compressed ambient air, closed, and sampled twice, first directly after closing then again after 4–5 h using a 35-mL syringe. The sample air was transferred to evacuated 12 mL glass vials (Exetainer, Labco Ltd. Lampeter, UK), first flushing the vials with 10 mL sample air using a second tip as exhaust, and subsequently inserting the remaining sample air with overpressure after removing the exhaust tip. Gas samples were analyzed with the GC-14B Analysis System TCD/FID and ECD (Shimadzu Corp., Kyoto, Japan). Gas emission rates were calculated using linear regression taking ambient temperature and the gas constant into account (Siegfried et al., 2011; Ingold, Khanal et al., 2018). Cumulative emissions were estimated using linear interpolation between sampling dates.

Volatilized ammonia was trapped in acidified glass fiber filters for 1 week after the manure application. Ammonia traps were modified from Ingold et al. (2018) by placing the acidified glass fiber filters on Teflon tape, which covered the opening of each polystyrol tube within the incubation containers. The Teflon tape did not affect the permeability for CO_2 and N_2O. Filters were acidified with 50 µL 1M $KHSO_4$ and incubation containers were closed air-tightly. Ammonia traps were exchanged after one week and placed in desiccators for three days for drying. Concentrated sulfuric acid was placed within the desiccator to trap ammonia in ambient air thus avoiding sample contamination. The dried filter samples were wrapped in tin capsules (IVA Analysetechnik GmbH & Co. KG, Meerbusch, Germany) and analyzed for N contents using a μEA elemental analyzer (CE Instruments, Rodano, Milano, Italy) coupled to a Delta Plus Isotope Ratio Mass Spectrometer through a Conflo III interface (Thermo Finnigan MAT GmbH, Bremen, Germany). The weekly cumulated ammonia emissions were calculated in relation to the unfertilized control representing the NH_3 volatilization potential.

TABLE 3 Mean pH, SOC, total N, K_2SO_4 extractable C and N, mineral N, net-N mineralization, and microbial biomass C (MBC) of incubated soils 30 days after manure application (M: Tannin-free manure, T: Tannin-enriched manure, and Tw; washed tannin-enriched manure) in the preliminary experiment with standard deviations in parentheses and lowercase letters indicate significant differences between manure treatments

		Sandy acidic topsoil			Sandy alkaline topsoil			*p*-values		
		M	T	Tw	M	T	Tw	Soil (S)	Manure (M)	S × M
pH		5.67 (b)	5.94 (ab)	6.04 (a)	8.83 (b)	8.91 (a)	8.89 (a)	< 0.001	0.001	0.020
		(0.02)	(0.15)	(0.19)	(0.04)	(0.03)	(0.00)			
SOC	(mg g^{-1} soil)	2.78	2.90	2.70	6.43	6.61	6.69	< 0.001	0.452	0.331
		(0.28)	(0.24)	(0.12)	(0.18)	(0.18)	(0.35)			
Total N	(mg g^{-1} soil)	0.26	0.27	0.27	0.42	0.41	0.43	< 0.001	0.398	0.557
		(0.03)	(0.01)	(0.02)	(0.02)	(0.01)	(0.02)			
K_2SO_4 extractable	C (% of SOC)	1.47	1.36	1.35	1.34	1.31	1.20	0.074	0.206	0.749
		(0.19)	(0.15)	(0.17)	(0.11)	(0.07)	(0.06)			
	N (% of total N)	23.40 (a)	16.00 (b)	16.66 (b)	5.85 (a)	3.17 (b)	2.73 (b)	< 0.001	0.001	0.189
		(4.69)	(2.61)	(2.01)	(1.34)	(0.32)	(0.11)			
Mineral N	NH_4^+ (μg g^{-1})	1.13	1.22	1.25	1.79	1.24	1.58	0.061	0.506	0.319
		(0.51)	(0.16)	(0.15)	(0.55)	(0.15)	(0.61)			
	NO_3^- (μg g^{-1})	20.98 (a)	5.58 (b)	5.50 (b)	44.57 (a)	34.43 (b)	33.21 (b)	< 0.001	< 0.001	0.447
		(7.17)	(0.92)	(0.26)	(2.86)	(6.24)	(3.08)			
Net-N mineralization	(μg kg^{-1} 30 d^{-1})	0.13 a	−2.93 (b)	−2.96 (b)	−0.05 (a)	−2.38 (b)	−2.64 (b)	0.534	< 0.001	0.703
		(1.19)	(0.23)	(0.10)	(0.73)	(1.43)	(0.73)			
MBC	(μg g^{-1})	56.1	59.7	48.0	86.3	101.9	106.4	< 0.001	0.435	0.177
		(11.95)	(18.72)	(10.99)	(3.94)	(17.30)	(13.88)			

2.6 | Statistics

The results of the two incubation experiments are presented as arithmetic means with standard deviation (in tables) and standard errors (in figures). For treatment comparison a generalized linear mixed model was used with soil as subject, and manure treatment, soil, and their interactions as fixed effects followed by a pairwise comparison of means using the sequential Bonferroni adjustment. For comparison of the manuring effect within the different soils, a univariate ANOVA followed by a Tukey test (with equal variances) or a Games–Howell test (with unequal variances) were conducted.

3 | RESULTS

3.1 | Preliminary experiment

SOC, total N, and microbial biomass C were significantly higher in the sandy alkaline topsoil compared with the sandy acidic topsoil, whereas manure treatments did not have any significant effect (Table 3). While extractable C was similar in both soils without a manure treatment effect, extractable N was fourfold higher on the sandy acidic topsoil compared with the sandy alkaline topsoil. In addition, tannin-enriched manure significantly reduced extractable N by 32%–46%. Ammonium concentrations were not affected by soil and manure treatments, but NO_3^- was significantly lower by 73%–23% in both topsoils, when amended with tannin-enriched manure compared with tannin-free manure resulting in a more negative net-N mineralization. The comparison of washed and unwashed tannin-enriched manure did not result in significant differences for any of the measured parameters.

3.2 | Main experiment

3.2.1 | Soil parameters

Manure application significantly increased SOC and total N in the sandy acidic topsoil and the alkaline subsoil (Table 4). K_2SO_4 extractable C was significantly affected by soil, manure, and their interactions. While Quebracho tannins led to a 27% reduction of extractable C (% SOC) in the manure applied loamy subsoil, it did not have an effect in the two topsoils. K_2SO_4 extractable N was 40%–73% lower ($p < 0.05$) in all soils amended with tannin-enriched manure compared to tannin-free manure after three weeks of incubation (Figure 1a, d, and g). Similarly, NO_3^--N was by 18%–74% reduced in topsoils treated with tannin-enriched manure compared with tannin-free manure (Figure 1b, e, h). This reduction of NO_3^--N concentration by Quebracho tannins remained even after 63 days. The application of tannin-enriched manure significantly reduced NH_4^+ concentrations on the loamy alkaline subsoil compared with tannin-free manure,

TABLE 4 Mean SOC, total N, K_2SO_4 extractable C and N, and C/N ratios of incubated soils 21 days after manure application (C: control, M: Tannin-free manure, and T: Tannin-enriched manure) in the main experiment with standard deviations in parentheses and lowercase letters indicate significant differences between manure treatments

								K_2SO_4 extractable					
		SOC (mg g^{-1} soil)		Total N (mg g^{-1} soil)		Soil C/N ratio		C (% of SOC)		N (% of total N)		C/N	
Sandy acidic topsoil	C	1.85 (b)	(0.03)	0.23 (b)	(0.01)	8.1 (c)	(0.31)	1.17 (a)	(0.28)	25.4 (a)	(1.31)	0.4 (c)	(0.10)
	M	2.42 (a)	(0.17)	0.27 (a)	(0.02)	8.8 (b)	(0.18)	1.64 (a)	(0.06)	23.9 (a)	(1.01)	0.6 (b)	(0.04)
	T	2.75 (a)	(0.35)	0.29 (ab)	(0.04)	9.4 (a)	(0.14)	1.51 (a)	(0.28)	16.7 (b)	(3.04)	0.9 (a)	(0.06)
Sandy alkaline topsoil	C	6.56 (a)	(0.41)	0.43 (a)	(0.04)	15.3 (a)	(1.14)	1.13 (a)	(0.19)	6.4 (a)	(0.67)	2.7 (c)	(0.50)
	M	7.14 (a)	(0.65)	0.47 (a)	(0.04)	15.3 (a)	(0.23)	1.50 (a)	(0.23)	4.0 (b)	(0.17)	5.7 (b)	(0.74)
	T	6.83 (a)	(0.55)	0.44 (a)	(0.03)	15.5 (a)	(0.73)	1.32 (a)	(0.15)	1.7 (c)	(0.31)	12.3 (a)	(3.00)
Loamy alkaline subsoil	C	2.91 (b)	(0.05)	0.12 (b)	(0.01)	23.6 (a)	(1.85)	0.45 (b)	(0.14)	1.4 (b)	(0.27)	14.8 (a)	(4.55)
	M	3.78 (a)	(0.13)	0.17 (a)	(0.00)	21.7 (b)	(0.37)	0.78 (a)	(0.07)	4.6 (a)	(1.01)	6.4 (b)	(1.75)
	T	3.85 (a)	(0.31)	0.17 (a)	(0.01)	22.5 (b)	(0.54)	0.57 (b)	(0.07)	2.9 (b)	(0.38)	7.6 (b)	(0.76)
p-values	Soil (S)	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	
	Manure (M)	< 0.001		0.001		0.001		< 0.001		< 0.001		0.008	
	S × M	0.254		0.323		< 0.001		0.826		< 0.001		< 0.001	

there was no clear effect on NH_4^+ concentrations of the two topsoils (Figure 1c, f, i).

In the short term, tannin-enriched manure induced a higher net-N immobilization than control and tannin-free manure on both sandy topsoils, whereas on the loamy alkaline subsoil no effect occurred (Figure 2). In the incubation period 3–9 weeks after manure application, net-N mineralization was negative for all treatments in the sandy acidic topsoil, whereas in the sandy alkaline topsoil the tannin-enriched manure treatment showed significantly higher net-mineralization than the tannin-free manure treatment. Microbial biomass C was significantly affected by soil and manure treatment in both experiments ($p <$ 0.001), whereby effects in the manured soils were mostly higher than in unmanured control (Table 5). The difference between tannin-free manure and tannin-enriched manure was nonsignificant. During the incubation experiments the pH value decreased or was unchanged in both sandy soils, when no manure or tannin-free manure was applied (Figure 3). In contrast, pH values with tannin-enriched manure in these soils slightly increased. In the loamy alkaline subsoil pH was slightly reduced and not affected by manure treatments.

3.2.2 | Gaseous emissions

The CO_2 emission rates averaged 0.13, 0.66, and 0.09 mg h^{-1} kg^{-1} from the unamended sandy acidic topsoil, the sandy alkaline topsoil, and the loamy alkaline subsoil, respectively. Average N_2O emission rates were 0.05, 0.03, and 0.01 mg h^{-1} kg^{-1}, respectively. Within the first 3 days after manure application, CO_2 emission rates in all 3 soils were significantly lower when mixed with tannin-enriched manure compared with tannin-free manure (Figure 4). In the subsequent days, CO_2 emission rates recovered to similar levels as in tannin-free manure treatments. Similarly, up to 3 days after manure application, N_2O emissions were 5.3–12.6-fold lower after tannin-enriched manure application compared with tannin-free manure application in all soils, but increased thereafter to higher emission rates in the two sandy topsoils. Cumulative CO_2 emissions were not significantly different for the two manure treatments in all soils. This was also true for cumulative N_2O emissions, except for 92% lower emissions in the tannin-enriched manure treatment of the loamy alkaline subsoil (Table 5).

Manure application resulted in maximum NH_3 volatilization of 0.05, 0.26, and 0.41 µg NH_3-N kg^{-1} soil h^{-1} in the sandy alkaline, sand acidic, and loamy alkaline soil, respectively (data not shown). While in the sandy alkaline topsoil no differences between manure treatments were observed, in the sandy acidic topsoil, tannin-enriched manure increased NH_3 volatilization potential numerically in both experiments by 15%–81 % (Table 5). In contrast, in the loamy alkaline subsoil NH_3 volatilization potential was 45%–58% lower ($p < 0.001$), when tannin-enriched manure was applied compared with tannin-free manure.

4 | DISCUSSIONS

4.1 | Net-N mineralization from manure

Our first hypothesis of a reduced net-N mineralization by ingested Quebracho tannins was supported by our results, except for the

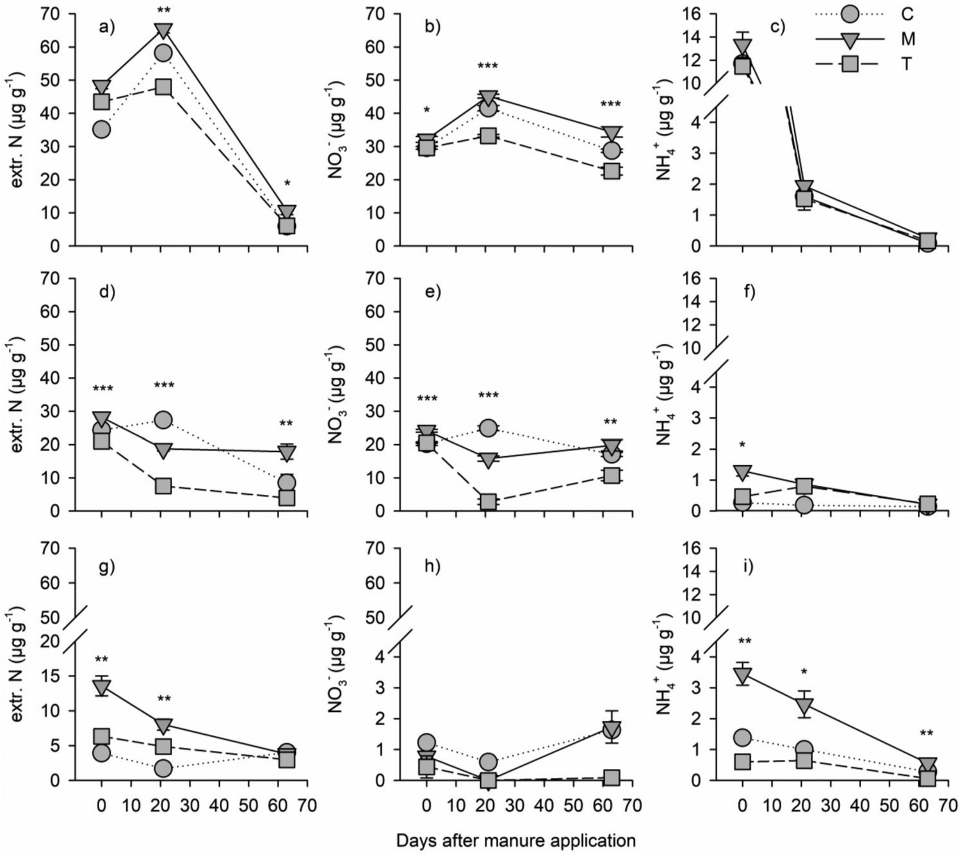

FIGURE 1 Mean concentrations of K_2SO_4 extractable N (a, d, and g), NO_3^- (b, e, and h) and NH_4^+ (c, f, and i) of the sandy acidic topsoil (a, b, and c), sandy alkaline topsoil (d, e, and f) and loamy alkaline subsoil (g, h, and i) incubated without manure (C) or with tannin-free manure (M) or tannin-enriched manure (T) during incubation of the main experiment (gray symbols) with standard errors indicated by whiskers. *X*-axes of extractable N and NO_3 concentrations were adjusted with breaks to visualize 3–20 times lower concentration levels in the loamy alkaline subsoil. Stars indicate significant differences between M and T within a soil, with significance levels of $^+p < 0.05$, $^{++}$ $p < 0.01$, and $^{+++}$ $p < 0.001$ for the preliminary experiment and respective * for the main experiment

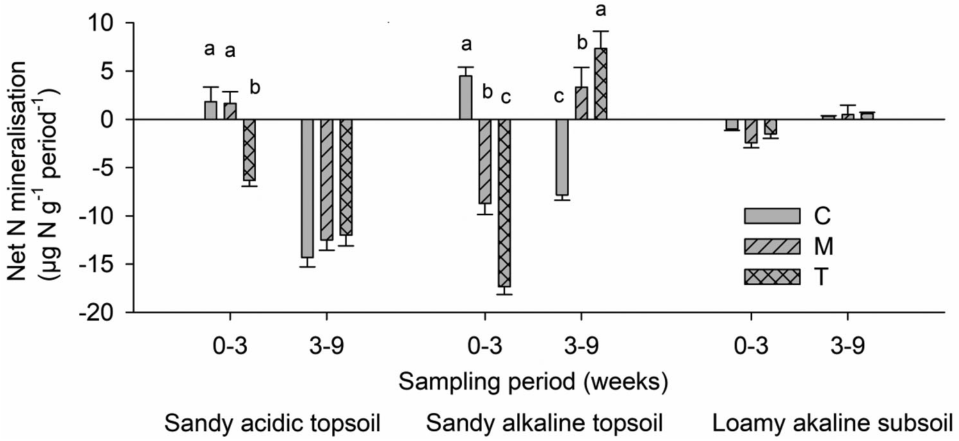

FIGURE 2 Net-mineralization during initial three weeks and subsequent 6 weeks in the main-experiment with standard errors indicated by whiskers. Letters indicate significant differences between manure treatments (C: control, M: Tannin-free manure, T: Tannin-enriched manure) within a soil

TABLE 5 Mean microbial biomass C (MBC) of incubated soils 0 and 21 days after manure application, NH_3 volatilization potential as % of unfertilized control, and cumulative CO_2 and N_2O emissions in the main experiment with standard deviations in parentheses. Lowercase letters indicate significant differences between manure treatments (C: control, M: Tannin-free manure, and T: Tannin-enriched manure)

		MBC (µg g^{-1})				NH_3 volatilization potential (% of unfertilized control)				Cumulative CO_2		Cumulative N_2O	
		0 days		21 days		Week 1		Week 2		(mg kg^{-1} 30 d^{-1})		(mg kg^{-1} 30 d^{-1})	
Sandy acidic topsoil	C	34.1 (b)	(14.55)	42.9 (b)	(19.23)					72.7 (b)	(13.75)	17.0 (b)	(3.93)
	M	102.7 (a)	(29.05)	95.4 (a)	(12.94)	77.2	(21.86)	101.6 (b)	(26.63)	766.5 (a)	(49.29)	945.4 (a)	(170.73)
	T	67.5 (ab)	(29.55)	78.4 (a)	(20.03)	92.0	(29.13)	184.2 (b)	(28.48)	805.0 (a)	(51.45)	1416.0 (a)	(689.82)
Sandy alkaline topsoil	C	31.4 (b)	(16.59)	93.0 (b)	(45.24)					500.4 (b)	(176.87)	12.6 (b)	(11.54)
	M	153.9 (a)	(34.89)	129.4 (ab)	(35.65)	126.7	(12.89)	107.5 (b)	(9.79)	2731.5 (a)	(116.88)	1369.6 (a)	(397.02)
	T	142.8 (a)	(22.53)	170.0 (a)	(19.95)	131.1	(45.79)	104.7 (b)	(14.18)	2810.4 (a)	(137.74)	1029.6 (a)	(185.84)
Loamy alkaline subsoil	C	20.4 (b)	(6.14)	29.1 (b)	(19.50)					71.4 (b)	(14.21)	0.0 (b)	(9.31)
	M	114.7 (a)	(51.40)	79.5 (a)	(19.87)	140.7	(27.55)	221.0 (a)	(11.35)	1559.3 (a)	(140.30)	222.1 (a)	(161.60)
	T	50.1 (ab)	(28.68)	68.9 (a)	(20.48)	77.8	(8.28)	91.9 (b)	(10.06)	1298.2 (a)	(230.98)	17.6 (b)	(6.72)
p-values	Soil (S)	0.001		< 0.001		0.247		0.037		< 0.001		< 0.001	
	Manure (M)	< 0.001		< 0.001		0.582		0.291		< 0.001		< 0.001	
	S × M	0.039		0.194		0.312		< 0.001		< 0.001		< 0.001	

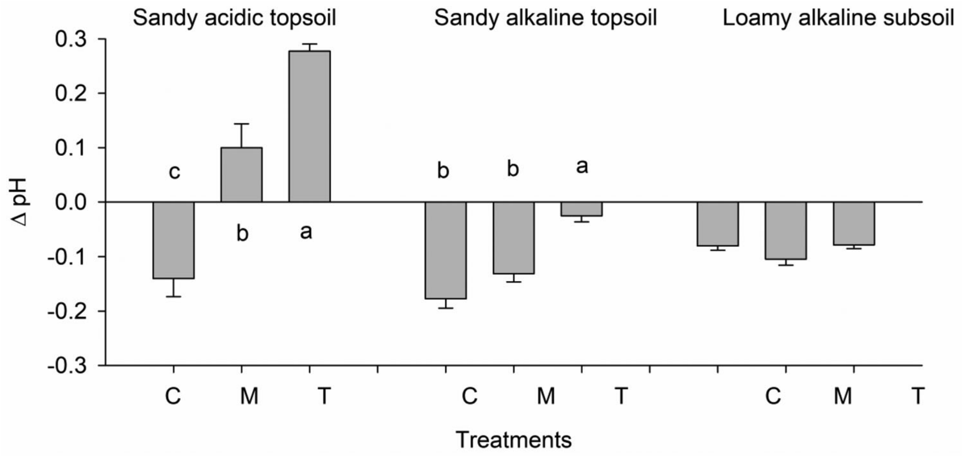

FIGURE 3 Changes of pH after three weeks of incubation in main experiment with standard errors indicated by whiskers. Letters represent significant differences between manure treatments (C: control, M: Tannin-free manure, T: Tannin-enriched manure) within a soil and time interval

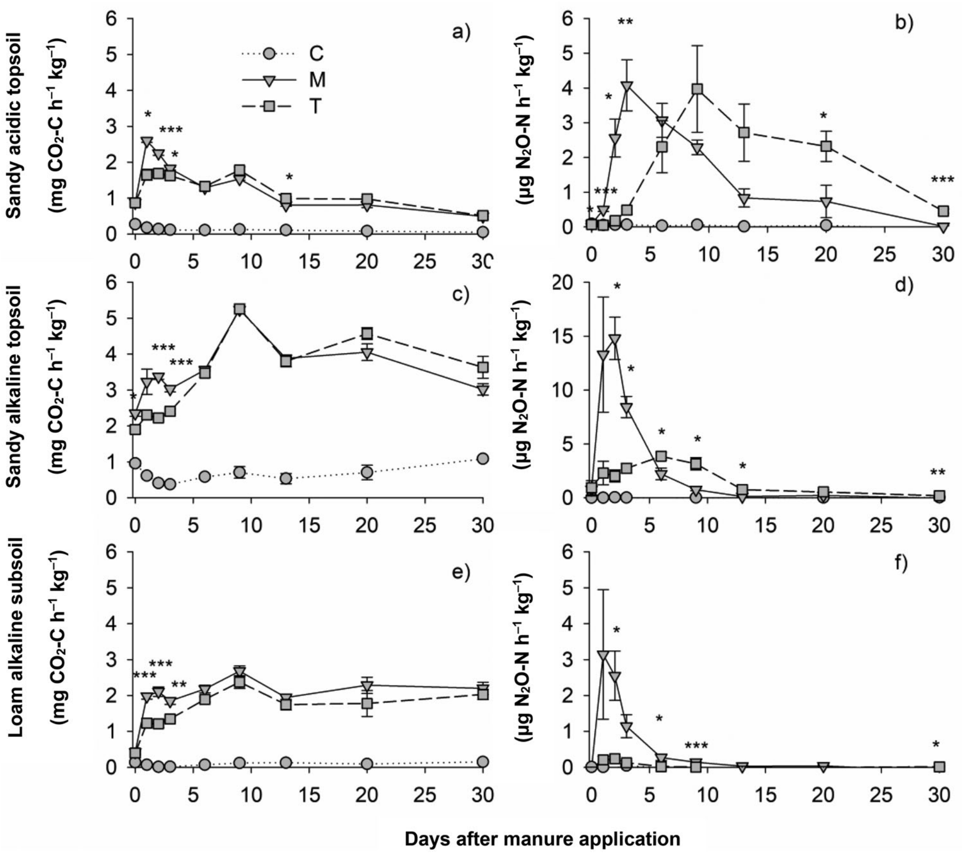

FIGURE 4 Mean emission rates of CO_2 (a, c, and e) and N_2O (b, d, and f) from the sandy acidic topsoil (a and b), sandy alkaline topsoil (c and d), and loamy alkaline subsoil (e and f) measured during main experiment after application of control (M), or tannin-enriched (T) with standard errors indicated by whiskers. Scale of *y*-axis of N_2O emission rates from the sandy alkaline soil was adjusted to fit the four to five times higher emission rates of the other two soils. Asterisks indicate significant differences between M and T within a soil, with significance levels of $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

contrasting loamy alkaline subsoil. In the two topsoils amended with tannin-enriched manure, mineral N strongly declined during the first 3–4 weeks of incubation compared to the tannin-free manure treatment. A similar result was found by Nierop et al (2006) when they investigated condensed tannins from different tree litter incubated in soil. Also, tannin-enriched manure used in a litterbag experiment on an irrigated sandy vegetable field in Oman released 36%–90% less N compared with tannin-free manure under sweet corn (*Zea mays* convar. *saccharata* var. *rugosa*) and radish [*Raphanus raphanistrum* subsp. *sativus* (L.) Domin] cultivation (Ingold et al., 2017), which resulted in lower N uptake and yields of the respective cultivated crops (Ingold et al., 2015). Frimpong et al. (2014) reported lower NO_3^- and NH_4^+ concentrations in a sandy loam soil incubated for 30 days with cowpea [*Vigna unguiculata* (L.) Walp.] residues, when either ferulic, vanillic, or tannic acid as polyphenol components were added. For tannic acid, this net-N immobilization was explained by a decreased mineralization due to protein complexation (Mutabaruka et al., 2007), whereas for the low molecular ferulic and vanilic acid stimulation of microbial activity was likely due to N immobilization (Frimpong et al., 2014). However, whether tannin-enriched manure reduces net-mineral N contents due to an inhibition of mineralization processes or an enhanced microbial activity has not been reported.

In both incubation experiments, the microbial biomass did not increase within 3 and 4 weeks of incubation and was not significantly affected by manure treatments; therefore, it is unlikely that N was immobilized by microbial activity. This was, however, not directly quantified in our study due the low levels of microbial biomass N. Thus, our results indicate that manure amendment with and without tannins did not stimulate microbial biomass in the short term. Soil respiration increased within the first three days after application of tannin-free manure. However, application of tannin-enriched manure initially reduced respiration but then increased thereafter to similar levels as tannin free manure. Thus, soil microorganisms were only shortly inhibited in their activity by ingested Quebracho tannins. Therefore, reduced K_2SO_4 extractable N and NO_3^- in soils manured with tannin-enriched manure cannot be explained by microbial N immobilization. Our analysis showed that about 15% of the extractable tannins and 6% of extractable condensed tannins in tannin-enriched manure were water-soluble. However, removal of water-soluble tannins by washing did not change the clear effects of tannin-enriched manure on net-N immobilization and reduced extractable N and NO_3^-. Because of their strong affinity to bind proteins, tannins affect N digestibility of animals, shifting N excretion from urine to feces, and fecal N excretion from labile to more stable and insoluble forms (Al-Kindi et al., 2016; Powell et al., 2009; Waghorn, 2008). The poorer ability to extract tannins from tannin-enriched manure compared with the ability to extract tannins from original tanniniferous feedstuff with standard analytical procedures indicates that tannins are bound to manure components, are chemically altered, or are degraded during digestion (Degen et al., 1995; Terrill et al., 1994). In line with our findings, Mutabaruka et al. (2007) found that soil incubated with Quebracho tannins-protein complexes had a lower net C and N mineralization than the sole application of either Quebracho tannin or proteins. Thus, the reduced net-N mineralization may rather result from a deprivation of N compounds by physico-chemical complexation by tannins (Adamczyk et al., 2017).

4.2 | Manure effects on gas emissions

The CO_2 and N_2O emission rates of the two unmanured soils were low and increased shortly after manure application, due to the input of easily available C and N sources in both manures. Overall, gaseous N and C losses were not consistently reduced in tannin-enriched manure treatments compared with tannin-free manure treatments. Also, the effect of Quebracho tannins varied over time and between the three soils. An explanation for this could be that tannins are known for their antimicrobial properties in soils and compost (Jordan et al., 2015) although their inhibitory effect was not always observed (Nierop et al., 2006). But in the current study, CO_2 emissions, originating from microbial respiration, were significantly reduced in all three soils directly after manure application, due to direct inhibition of soil microorganisms or a reduction in substrate availability (Hättenschwiler & Vitousek, 2000; Kraus et al., 2004). Despite this, the microbial activity increased thereafter to similar emission rates as from tannin-free manured soils, which contradicts our second hypothesis. A plausible explanation is that the initially inhibited microorganisms recovered and overcame possible antimicrobial tannin effects and or substrate deprivation. It has been reported that Quebracho tannins shift microbial community composition in feces and soil from bacteria towards fungi dominated communities (Al-Kindi et al., 2016; Mutabaruka et al., 2007; Sradnick et al., 2014). Some saprotrophic fungi might be less affected by tannin-enriched manure, as they were shown to utilize protein-tannin complexes (Bending & Read, 1996). However, whether microorganism traits were able to overcome inhibitory effects of Quebracho tannins merits further investigation.

N_2O is a product of biological processes, such as anaerobic denitrification, and autotrophic and heterotrophic nitrification processes (Butterbach-Bahl et al., 2013). Besides soil physico-chemical soil properties, N_2O emissions from soils are controlled by N and C substrate availability (Butterbach-Bahl et al., 2013). This was shown by the high correlation of the basal N_2O emissions from the three soils with the availability of extractable N and NO_3^-. N_2O emissions were also strongly inhibited by tannin-enriched manure during initial 3 days but increasing thereafter to delayed peaks on the sandy acidic topsoil and the sandy alkaline topsoil. On the sandy acidic topsoil incorporated with the tannin-enriched manure the N_2O emission rate reached the same level as the same soil incorporated with tannin-free manure, whereas on the sandy alkaline soil tannin-enriched manure lowered the maximum N_2O emission rate compared with tannin-free manure. Decreased N_2O emissions due to a lower availability of inorganic N were reported for cowpea residue mixed with polyphenols (Frimpong et al., 2014) and tannin-containing legumes (Millar & Baggs, 2004). Therefore, the reduction of extractable N and NO_3^- by tannin-enriched manure serves as an explanation for the initial strong inhibition of N_2O emissions. The effect of tannin-enriched manure on subsequent N_2O emissions differed between the three soils, and this may be due to the different substrate contents for nitrification (Butterbach-Bahl et al., 2013). The inhibitory effect of Quebracho tannins reverted in soils with a higher availability of inorganic N, indicating that microorganisms utilized N from the soil pool when N from manure was less available. However, on the two alkaline soils N_2O emissions from tannin-enriched manure never reached levels of tannin-free manure. This suggests a possible influence of soil pH on inhibitory Quebracho tannin effects. On the one hand, pH affects the formation and degradation of tannin-protein complexes (McNabb et al., 1998), on the other hand phenol oxidizing enzyme activity is influenced by pH.

In both alkaline soils initial NH_4^+ concentrations were low, since on alkaline soils NH_4^+ is usually deprotonated quickly and lost via NH_3 volatilization. However, the ammonia volatilization potential was not affected by manure treatments in the sandy alkaline topsoil, whereas in the loamy alkaline subsoil it was significantly reduced by 45%–60% in the tannin-enriched manure treatment. In other studies tannins were found to reduce NH_3 volatilization from barn floors and soil amended with cow slurry by 28 to 49% (Hess et al., 2006; Powell et al., 2011a, 2011b). The main source of NH_3 volatilization in these studies was urea and a reduced volatilization was attributed to a reduction of urea excretion and an inhibition of urease activity by tannins. Also in feces, NH_3 volatilization was found to correlate with total ammoniacal N concentration (Misselbrook et al., 2005), which would explain the reduced NH_3 volatilization potential of the loamy alkaline subsoil during the first sampling period. In contrast, NH_3 volatilization from the sandy moderate acidic topsoil was consistently, though not significantly, higher with tannin-enriched manure application compared with tannin-free manure application. Furthermore, the increased ammonia volatilization potential in the tannin-enriched manure applied soil corresponded with higher soil pH on the sandy moderate acidic soil in both incubation experiments. This corresponding increase is supported by Martens and Bremner (1989) who found that NH_3 volatilization from soil applied urea positively correlated with the soil pH after incubation due to deprotonation of ammonium with rising pH. Although changes in soil pH were small with increases from initially 5.7 of 0.04–0.28 pH units, pH effects at microsite scale might have been much larger.

5 | CONCLUSIONS

The clear reduction in initial net-mineralization, extractable N and NO_3^-, and initial N_2O emission rates demonstrated the relevance of tannins in animal feed on N turnover processes in manured soils. However, addition of Quebracho tannins did not generally inhibit microbial activity and mineralization processes in manured soils, when ingested by animals. The two investigated topsoils, differing in pH, SOC, and K_2SO_4 extractable N contents showed consistent effects of tannin-enriched manure on initial net-mineralization, extractable N and NO_3^-, as well as gaseous CO_2 and N_2O emissions. However, NH_4^+, NH_3 volatilization potential, and net-mineralization three to nine weeks after manure application showed divers effects for the two topsoils. The contrasting loamy alkaline subsoil differed in its reaction of NH_4^+ concentration, net-N mineralization, pH changes, NH_3 volatilization potential, and cumulative N_2O to tannin-enriched manure from the other two soils. This indicated that varying soil properties affected soil N-dynamics differently in the course of a 9-week incubation experiment and illustrates the need of long-term experiments with different soils to evaluate the effects of integrating tannins into livestock diets on the agricultural environment. Our results show that tannin-enriched manure can reduce greenhouse gas emission rates and NH_3 volatilization potential under laboratory conditions, but this effect depends on soil type. The initial immobilization followed by a net-mineralization on the sandy alkaline topsoil amended with tannin-enriched manure, may help to adapt N release from manure to crop demand under irrigated subtropical conditions. The clear reduction in soluble N and particularly NO_3^- in the three soils amended with tannin-enriched manure illustrated the potential of tannins in animal diets to mitigate N leaching losses in soils.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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