Science of the Total Environment 784 (2021) 147139

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Slow pyrolysis liquid in reducing NH_3 emissions from cattle slurry — Impacts on plant growth and soil organisms

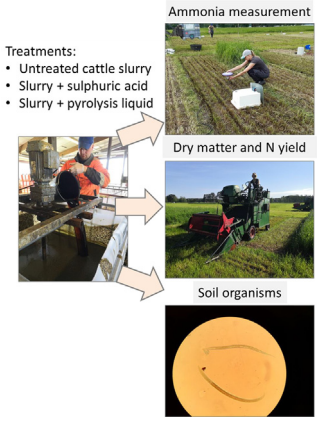
Marleena Hagner [a,b,*], Mari Räty [c], Johanna Nikama [a], Kimmo Rasa [a], Sari Peltonen [d], Jouko Vepsäläinen [e], Riikka Keskinen [a]

[a] Natural Resources Institute Finland, 31600 Jokioinen, Finland
[b] Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, 15140 Lahti, Finland
[c] Natural Resources Institute Finland, 71750 Maaninka, Finland
[d] Association of ProAgria Centres, 01301 Vantaa, Finland
[e] University of Eastern Finland, Faculty of Health Sciences, School of Pharmacy, 70211 Kuopio, Finland

HIGHLIGHTS

- Ability of pyrolysis liquid to reduce NH_3 emissions of cattle slurry was studied.
- Pyrolysis liquid (PL) acidified slurry reduced NH_3-N emission rate remarkably.
- Plant germination and yield was reduced after the application PL acidified slurry.
- No negative effect on soil nematodes and enchytraeids were found.
- PL containing organic compounds were rapidly degraded by soil microbes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 November 2020
Received in revised form 9 April 2021
Accepted 10 April 2021
Available online 16 April 2021

Editor: Charlotte Poschenrieder

Keywords:
Acidification
Grass ley
Acetic acid
Pyroligneous acid
Wood vinegar
Sulfuric acid

ABSTRACT

A substantial percentage of manure nitrogen (N) can be lost as gaseous ammonia (NH_3) during storage and field spreading. Lowering slurry pH is a simple and accepted method for preserving its N. Efficiency of slow pyrolysis liquid (PL) produced from birch (*Betula* sp.) as an acidifying agent, and its ability to reduce NH_3 emissions following surface application of cattle slurry, was studied in a field experiment. Untreated slurry (US) and slurries acidified with PL and sulfuric acid (SA) were applied to the second harvest of a grass ley. Immediate NH_3 emissions, grass biomass, N-yield and possible toxic impacts on soil nematodes and enchytraeids were examined. Furthermore, the effects on soil respiration, nitrogen dynamics and seed germination were studied in subsequent laboratory experiments.

In the field, over one third of the water-extractable ammonium-N (NH_4-N) applied was lost through NH_3 volatilization from US. SA and PL acidified slurries reduced NH_3-N emission rate equally from 3.4 to <0.04 kg ha^{-1} h^{-1}. Acidification with SA resulted in the highest and that with PL in the lowest grass dry matter (DM) and N yield. Neither SA nor PL acidification had negative effects on soil enchytraeids or nematodes. Reduced yield production, seed germination and delayed microbial activity after PL slurry application were most probably caused by the PL containing organic compounds. However, later increase in carbon dioxide (CO_2) production and improved seed

* Corresponding author at: Natural Resources Institute Finland, 31600 Jokioinen, Finland.
E-mail address: marleena.hagner@luke.fi (M. Hagner).

https://doi.org/10.1016/j.scitotenv.2021.147139

germination suggest that these compounds were rapidly volatilized and/or degraded by soil microbes. Though PL efficiently cut NH_3 emission from surface-spread slurry, further studies on appropriate application methods and possible phytotoxicity are needed.

1. Introduction

Global agriculture produces annual emissions of, on average, 32 Tg ammonia-nitrogen (NH_3—N), of which a major share derives from animal manures (Beusen et al., 2008). Volatilized NH_3 contributes to acidification and eutrophication of the immediate environment (Kanter, 2018; McCubbin et al., 2002; Tang et al., 2021) and impairs air quality, thus compromising animal and human welfare (Giannadaki et al., 2018). Furthermore, the loss of gaseous N decreases the fertilizer value of manure, causing economic loss for the farmer and hampers evaluation of the realized N application rate. By preventing NH_3 emissions, the manure N could be preserved for crop growth and consequently application of synthetic N fertilizers could be reduced (Kai et al., 2008). Ultimately, cutting NH_3 emissions would benefit agricultural producers, the environment, and the whole of society.

Most manure N is bound to organic compounds from which it is released as ammonium (NH_4^+) ions through mineralization. The aqueous NH_4^+ is in balance with gaseous NH_3 so that in acidic conditions only NH_4^+ exists whereas in alkaline conditions the balance is towards NH_3 (Hartung and Phillips, 1994). In animal slurries, pH is naturally high, which explains their susceptibility to NH_3 emissions (Sommer and Hutchings, 2001). Lowering the slurry pH below 6 represents a simple method for preserving its N. Reported NH_3 emission reductions achieved with slurry acidification during storage and field spreading range from 10 to 100% though the highest reductions have been recorded in laboratory studies (Fangueiro et al., 2015; Ndegwa et al., 2008). Different acidifying additives, e.g. strong mineral acids, weak organic acids and various salts have been tested for the pH adjustment but in practice sulfuric acid (H_2SO_4, SA) is the most commonly applied (Ndegwa et al., 2008; Fangueiro et al., 2015). Though favored for its efficiency, handling of concentrated SA is hazardous at the farm level and alternative agents are thus desired (Fangueiro et al., 2015).

Recently, Keskinen et al. (2018) suggested pyrolysis liquids (PLs) to be potential candidates for the acidification technology. Pyrolysis liquids are often considered by-products in the thermochemical treatment applied for converting various biomasses to gas for energy and char for different purposes (Libra et al., 2011). The PLs are aqueous solutions that contain a wide range of organic compounds, of which acetic acid is the most common, followed by methanol, hydroxyacetone, formic acid, furfural and propanoic acid in variable order depending on the feedstock material and the process technology (Fagernas et al., 2012; Hagner et al., 2020). The total acidity of PLs ranges from 850 to 2560 meq l^{-1}, and the application rates in slurry acidification rise to tens of liters per tonne (Keskinen et al., 2018). However, the use of PLs could improve the safety and ecological sustainability of the acidification treatment as the total acidity of PLs consists of weak organic acids and the pyrolysis process promotes circular economy by exploiting side-stream biomasses.

Considering safety and the environment, occurrence of potentially harmful elements (e.g. aldehydes, furans and polycyclic aromatic hydrocarbons) in the PLs needs to be accounted for (Diebold, 1997). Supporting the possible agronomic use of PLs, they have been shown to be readily biodegradable (Campisi et al., 2016) and only slightly toxic to soil organisms (Hagner, 2013). Nonetheless, manipulation of the pH, as such, impacts multiple chemical and microbial processes in the slurry (Fangueiro et al., 2015). The effects of further changes in the slurry composition brought about by the PL addition on microbe activity, usability of nutrients, plant growth and soil biology are required before introducing the method for public use.

In this study, we investigated the usability of PL derived from pyrolysis of birch wood (*Betula* sp.) in reducing the NH_3 emissions during short-term storage and surface application of cattle slurry in a field experiment with a perennial grass mixture. The effects of PL treated slurry on plant growth and soil nutrient status were examined in comparison with untreated and SA acidified slurries. Further, possible toxic impacts of the treatments were examined through observing nematodes and enchytraeids, which are key organisms in the soil, participating in the soil nutrient cycle by releasing nutrients by breaking down organic material and improving soil structure (Bardgett, 2005). Abundance of nematodes and enchytraeids promptly responds to disturbances in soil and the response of bacterial- and fungal-feeding nematodes can be used as an indicator of microbial growth (Christensen et al., 1992; Mikola and Setälä, 1998; Sohlenius, 1990). To explain the results obtained in the field, additional studies were carried out in the laboratory. These experiments clarified the effects of the slurry treatments on soil respiration, N dynamics and seed germination.

Based on earlier knowledge of SA usability in slurry acidification, PL chemical properties and laboratory scale indications of PL performance in acidification, we hypothesized that: (1) SA and PL reduce NH_3 emissions comparatively, (2) PL increases plant growth and N yield equally to SA, (3) PL induces no effect on soil enchytraeids and nematodes, and (4) PL is rapidly consumed by soil microbes as indicated by increased microbial activity.

2. Materials and methods

2.1. Field experiment

2.1.1. Study site

The field study was conducted on the premises of the Natural Resources Institute Finland in Maaninka, Kuopio, in east-central Finland (63°8′N, 27°18′E). Soil in the experimental field was Humic Dystric Regosol (IUSS Working Group WRB, 2015). According to texture, the topsoil (0–20 cm) was loam (clay 9%, silt 47%, sand 44%) and the subsoil (20–40 cm) sandy loam (clay 7%, silt 48%, sand 45%). Organic carbon and total N contents were 1.6% and 0.1% in the topsoil, and 1.2% and 0.1% in the subsoil, respectively. Potential cation exchange capacity (1 M CH_3COONH_4, pH 7.0) was 11.7 cmol(+) kg^{-1} in the topsoil and 10.5 cmol(+) kg^{-1} in the subsoil and pH(H_2O) 6.1 and 6.3, respectively. During previous years, the study area had been under autumn-ploughed cereal cultivation with NPK compound fertilization and had not received organic fertilizers. Based on agronomic soil testing (0.5 M acid ammonium acetate at pH 4.65 by Vuorinen and Mäkitie, 1955), the status of phosphorus (P) and potassium (K) was satisfactory and that of calcium (Ca) and magnesium (Mg) fair (17.0 mg P l^{-1}, 181 mg K l^{-1}, 1335 mg Ca l^{-1} and 104 mg Mg l^{-1} in the topsoil layer). For determination of the bulk density and soil moisture, soil profile samples were taken by window-type augers of 4.8 cm and 2.3 cm in inner diameter, respectively, to a depth of 20 cm, and sliced into 5 cm segments. The bulk density and mass wetness were determined gravimetrically by drying at 105 °C for 48 h. For the soil layers of 0–5, 5–10, 10–15 and 15–20 cm, the dry bulk density averaged 1.13, 1.26, 1.50 and 1.58 g cm^{-3}, respectively. The dry bulk density was used to convert gravimetric soil water content into volumetric water content.

Daily air temperature and precipitation during the field experiment were obtained from the Finnish Meteorological Institute's observation station located at Luke Maaninka, and long-term averages for the normal period covering the years 1981 to 2010 from Pirinen et al. (2012) (Supplementary Fig. 1). Soil moisture, i.e. matric suction, was monitored in the experimental area during the growing season with tensiometers (Irrometer® IR-45, IR-60, IR-90) at depths of 15, 40 and 65 cm with 2–4 replicates. Compared with the long-term average of 218 mm, the summer 2019 (June–August) was unusually dry with a monthly precipitation sum of 95 mm, whereas the autumn was characterized by wet conditions (Supplementary Fig. 1). The period with low rainfall resulted in soil drying to the depth of 40 cm from mid-July to mid-August, whereas at the depth of 65 cm, soil moisture remained unchanged over the growing season (Supplementary Fig. 2).

2.1.2. Experimental design

The field experiment was conducted in a randomized complete block design with four replicates and a plot size of 12 m^2 (1.5 m × 8 m) in 2019. The grass ley was established on May 24 in 2018 with a mixture of timothy (*Phleum pratense* L., cv. Nuutti, seed rate 14 kg ha^{-1}) and meadow fescue (*Festuca pratensis* Huds., cv. Valtteri, seed rate 10 kg ha^{-1}), using barley (*Hordeum vulgare* L.) as the cover crop (cv. Toria). In the year of establishment, the study area was fertilized uniformly according to the Finnish recommendations using N, P and K inorganic fertilizers (75–17–25 kg ha^{-1} NPK). On May 10, 2019, all the plots were fertilized similarly for the first harvest, with 100 kg N ha^{-1}, 0 kg P ha^{-1}, 28 kg K ha^{-1} and 16 kg sulfur (S) ha^{-1}. The experimental treatments were implemented for the second harvest immediately after the first harvest on June 17. For the third harvest, collected to estimate the residual effect of the treatments, no fertilization was applied.

The experiment involved three treatments with dairy cattle slurry: 1) untreated slurry, 45 t ha^{-1} (US), 2) slurry acidified with sulfuric acid, 45 t ha^{-1} (slurry + SA) and 3) slurry acidified with pyrolysis liquid (slurry + PL), 49 t ha^{-1}. The respective dry matter (DM) contents of slurries were 7.8%, 6.8% and 7.0%. The slurries were surface applied by watering cans in bands at intervals of 0.3 m, representing the band spreading technique. For obtaining a crop N response curve, three treatments receiving increasing mineral N levels (complemented with P and K) were included: 1) unfertilized control receiving no added N, 2) 40 kg N_{min} ha^{-1} and 3) 70 kg N_{min} ha^{-1}. The mineral fertilizers were broadcasted using a Tume (RL 1500) spreader. Every other plot was left as a safety zone between the treatment plots (Fig. 1).

The application rates for slurries were estimated to deliver the same dose of water-extractable NH_4-N for the slurry treatments, and the same dose of P for all treatments (Table 1). However, despite the enlarged application rate of PL-acidified slurry calculated to compensate for the dilution effect caused by the PL volume, the realized amount of soluble N in the PL treatment lagged behind that in the US and SA. The total N content in the US and slurry + SA at the time of application was 3.6 g kg^{-1}, whereas in the slurry + PL the corresponding value was 2.9 g kg^{-1}. The easily available, water extractable NH_4-N contents were 1.4, 1.5 and 1.2 g kg^{-1} in the US, SA and PL slurries, respectively. For total P, the contents were 0.55 (US), 0.54 (slurry + SA) and 0.46 (slurry + PL) g kg^{-1}. The slurry S content increased from 0.4 to 3.0 g kg^{-1} due to the SA addition, resulting in a high application rate of 133 kg S ha^{-1} as compared to that of 16–19 kg S ha^{-1} for other slurry treatments. The K and S additions were not adjusted equally among the treatments. In the same experimental field, soil S status has remained in the classes of fair-satisfactory (6–15 mg S l^{-1}), and moreover, no grass yield response to S fertilization was registered in a previous study (Hyrkäs and Virkajärvi, 2014). As for K, the differences between treatments were relatively small. The total K content in the US and slurry + SA was 3.9 g kg^{-1} and in the slurry + PL 3.3 g kg^{-1}.

2.1.3. Acidification procedure

Commercially available concentrated SA (KemAcipro TECH™ 93%; density of 1.82 kg dm^{-3}) and PL (Charcoal Finland Ltd.) were used as the acidifying agents. The PL derived from birch wood (including bark) that was pyrolysed in a batch retort at maximum temperature of 450 °C held for 2 h. Total C content (TOC) of the PL was 20.0%, N 0.09% and pH 2.8. Contents of other main components in the PL were analyzed in the University of Eastern Finland using Nuclear Magnetic Resonance (NMR) (Table 2). Polyaromatic hydrocarbons (PAHs) were analyzed at Eurofins Nab Labs Ltd. Total sum of 16 EPA PAH was 1.3 mg kg^{-1}.

Cattle slurry with a dry matter content of 7.8% was collected from a conventionally operating dairy farm of the Natural Resources Institute

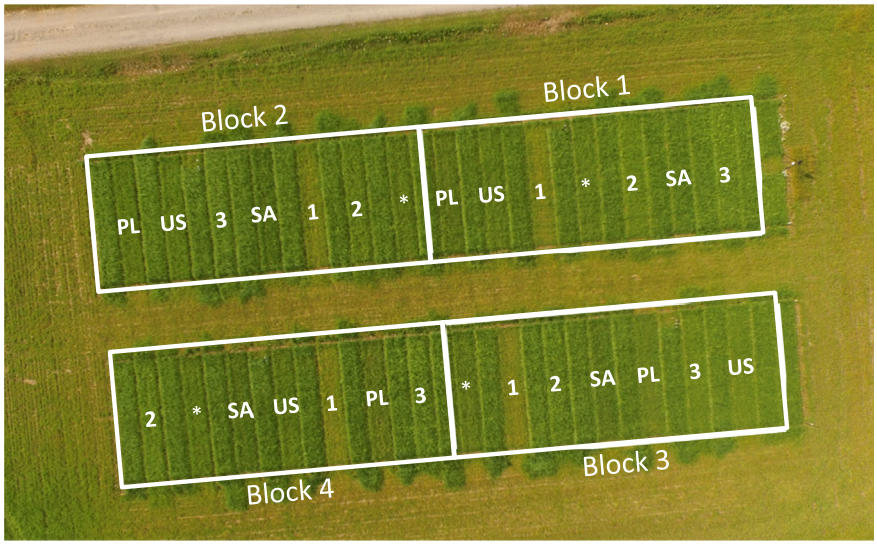


Fig. 1. Experiment consisted of four blocks, each including three treatments with dairy cattle slurry: 1) untreated slurry, 45 t ha^{-1} (US), 2) slurry acidified with sulfuric acid, 45 t ha^{-1} (slurry + SA) and 3) slurry acidified with pyrolysis liquid (slurry + PL), 49 t ha^{-1}. For obtaining a crop N response curve, three treatments receiving increasing mineral N levels (complemented with P and K) were included: 1) unfertilized control receiving no added N, 2) 40 kg N min ha^{-1} and 3) 70 kg N min ha^{-1}. Every other plot was left as a safety zone between the treatments. Data from one treatment marked as * is not shown in this paper.
Photo by Atte Kiiski (ProAgria Eastern Finland).

Table 1
Application rates (kg ha^{-1}) of total nitrogen (N), water-extractable ammonium nitrogen (NH_4-N), total phosphorus (P) and total potassium (K) for the 2nd harvest of grass ley (average ± standard deviation, n = 3). 0 N_{min} = unfertilized control receiving no added N, 40 N_{min} = 40 kg N ha^{-1} and 70 N_{min} = 70 kg N_{min} ha^{-1}, applied as inorganic N fertilizers. US = untreated dairy cattle slurry, slurry + SA = dairy cattle slurry acidified with sulphuric acid and slurry + PL = dairy cattle slurry acidified with pyrolysis liquid.

	Application rate (kg ha^{-1})[a]			
	Total N[b]	Water extractable NH_4-N[c]	Total P[d]	Total K[d]
0 N_{min}	0	0	23	139
40 N_{min}	40	40	23	140
70 s	70	70	23	142
US (45 t ha^{-1})	164 ± 4.1	62 ± 2.0	25 ± 0.5	176 ± 3.4
Slurry + SA (45 t ha^{-1})	164 ± 3.2	68 ± 4.2	24 ± 1.3	176 ± 1.1
Slurry + PL (49 t ha^{-1})	144 ± 11	58 ± 9.2	23 ± 1.6	162 ± 25

[a] Slurry samples were taken at the time of field spreading and determined in triplicate.
[b] Total concentration of N was analyzed by the Kjeldahl digestion method by boiling in concentrated H_2SO_4, followed by NH_3-N distillation and titration (Kemppainen, 1989).
[c] Water-extractable NH_4-N and NO_3-N was analyzed using 1:5 water extraction (EN 13652:, 2001) and determined colorimetrically with a Skalar San^{++} autoanalyzer (all inorganic N was in the form of NH_4-N).
[d] Total concentrations of P, K and S were analyzed by wet digestion with concentrated HNO_3 and determined with a ICP-OES (Perkin Elmer Optima 8300).

Finland in Maaninka. Required amount of slurry was drawn into a tractor-drawn tank on June 11, 2019 from a 100 m^3 covered storage pit serving as a pre-tank for a farm-scale biogas plant. After thorough mixing, the slurry was poured into three open 1 m^3 plastic containers. The containers were filled gradually one after another and weighed prior to and subsequent to the slurry addition. Finally, each container carried approximately 610 kg of slurry. One container was preserved untreated at the original pH (US), whereas the slurry in the other two containers was acidified either by SA or PL targeting at pH 5.0. Prior to the acid additions and pH measurements, the slurries were stirred for about 3 min with a container mixer (HLS 0.75/90, Mamec Oy, Finland) at 90 rpm. During the acidification, the stirring was gentler due to strong foam formation. The untreated slurry was stirred similarly to the acidified treatments. In the SA treatment, the total addition level was 4.9 l SA t^{-1} slurry (5.1 l m^{-3} slurry), in which SA was applied in two parts due to the need for pH readjustment on June 14. In the PL treatment, 83 l PL t^{-1} slurry (86 l m^{-3} slurry) was needed. Considerable foaming diminished the manageability of slurries during the day of acidification and next day. Following the acidification treatments, the containers were stored uncovered for six days before spreading on the experimental plots in an unheated storehouse, where temperature followed the temperature in the open air. The approach thus represented short-term storage tank acidification techniques. At the time of application, the slurry pH was 6.9, 5.1 and 5.3 in the US, slurry + SA and slurry + PL, respectively.

Table 2
Main components of birch pyrolysis liquid used in acidification analyzed by NMR and calculated concentrations of compounds ending on the soil during the application of pyrolysis liquid acidified slurry (49 t ha^{-1}, containing PL 83 l t^{-1}). Mixing depth of 5 cm, soil bulk density of 1500 g l^{-1} and PL specific weight of 0.98 g ml^{-1} were used in calculations producing 4067 kg of PL ha^{-1} i.e. 5314 mg kg^{-1}.

Compound	g l^{-1}	Concentration in the soil mg kg^{-1}
Acetaldehyde	0.00	0.00
Furfural	0.85	4.51
Hydroxymethylfurfural	0.00	0.00
Formic acid	0.16	0.84
Phenol	0.37	1.97
Catechol	2.67	14.2
1-Hydroxy-2-butanone	4.25	22.6
1-Hydroxy-2-propanone	25.0	133
Methanol	3.74	19.9
Methyl acetate	0.00	0.00
Acetic acid	98.8	525
Lactic acid	0.00	0.00
Ethanol	0.00	0.00
Propionic acid	4.01	21.3

2.1.4. Measurement of NH_3 volatilization

The NH_3 emissions were measured using the equilibrium concentration technique (JTI method), which combines the stirred dynamic chamber method with a passive diffusion sampler technique, allowing simultaneous measurements in small plots (e.g. Svensson, 1994; Mattila, 2006). Briefly, two different types of passive diffusion samplers were used for measuring NH_3 concentration in the air. In the L-type samplers, absorption filters were mounted at the top of the samplers, being directly exposed to the ambient air. In the C-type samplers, membranes (Fluoropore® PTFE, hydrophobic) were placed on the top and absorption filters at the bottom of the samplers. The absorption filter papers (Whatman® Grade 40) were impregnated with 2% oxalic acid ($C_2H_2O_4 \times 2\ H_2O$) – methanol (CH_3OH) – solution and dried in a desiccator. The samplers were mounted on the vertically adjustable sampler holders and placed inside and outside the chamber. A white-colored plastic container with a volume of 0.03 m^3 (length 0.40 m × width 0.32 m × height 0.20 m) was used as the chamber and equipped with a battery-operated air mixing fan. For excluding the influence of external wind, a metal plate was placed in front of the opening of the ventilated chamber.

In the surface-applied slurry treatments (US, SA, PL), the measurements of NH_3 volatilization were started in each plot immediately after spreading the slurry. A steel frame covering an area of 0.12 m^2, on top of which the chamber was then set, was placed on the ground over two slurry bands. The sampler holder was located between the bands. One ambient air sampler holder, equipped with plastic shelter against drizzle, was also placed between the slurry bands but about 1.5 m distant from the chamber. Each sampler holder consisted of a pair of both types of passive diffusion samplers. Background concentration was measured on three adjacent plots of grass leys that had not received organic manures. In total, five series of NH_3 measurements were conducted during three days following the slurry application. Two series of measurements were implemented on the first (June 17) and second day (June 18). These measurement periods ranged from an average of 103 to 143 min and 184 to 238 min, respectively. One series of measurements lasting 297 min was carried out on the third day (June 19).

For the determination of NH_4-N concentrations (mg l^{-1}), the filter papers were extracted with 8 ml of water for 30 min and the extracts measured with a Skalar San^{++} autoanalyzer (Skalar Analytical B.V., Netherlands). Calculations of NH_3 volatilization rate were made by applying equations as described in detail by e.g. Svensson (1994) and Mattila (2006).

During the period of NH_3 measurements, precipitation, temperature and wind speed data were recorded with a weather station (a-Weather, a-Lab Oy, Finland) at a sampling rate of 15 min. Wind speed was measured with a cup anemometer at 1.7 m height (Supplementary Fig. 3).

2.1.5. Above- and below-ground plant biomass

The grass ley plots were harvested to the height of 7 cm with a Haldrup plot harvester on June 17, August 9 and September 17. Fresh yield of grass was collected from an area of 12 m^2 and weighed with an onboard weighing system. Separate grass samples were collected for the analyses of dry matter (DM) content and determination of N contents from each plot. For calculating the total dry yield, DM content of the fresh grass samples was determined gravimetrically by drying at 60 °C for 48 h. Grass N content was determined by dry combustion (Leco® TruMac CN analyzer, LECO Corporation, USA).

To estimate the below-ground biomass, soil samples were taken on October 9 from the plant row with an auger (diameter 4.6 cm) at the

depths of 0–10 and 10–20 cm, with four replicates in each plot. The samples were frozen in plastic bags until organic material was separated from the soil using a hydropneumatic elutriation system (Smucker et al., 1982). Prior to the washing procedure, the frozen samples were thawed overnight at 4 °C, after which a volume of tap water corresponding to two thirds of the sample mass was added to each sample. Thereafter, the samples were left to soak overnight. After washing, roots were hand sorted from other organic debris. Total root dry mass, including living and dead roots, was determined by weighing after drying at 50 °C for 72 h. The root material was ground for total N, which was determined by dry combustion (Leco® TruMac CN analyzer).

2.1.6. Availability of soil N

Bulk soil samples were taken from each plot with an auger at the depths of 0–20 and 20–40 cm in the spring of 2019 (May 7), mixed and combined into one composite sample per replicate within each soil layer. After the third (October 9) harvests, soil samples were collected from each plot at the depths of 0–20 and 20–40 cm and frozen until N analysis. Soluble total N and inorganic N (NH_4-N and NO_3-N) were extracted with 2 M KCl (1:5 soil:solution ratio, 2 h), filtered and determined with a Skalar San^{++} autoanalyzer. Soluble organic N (SON) was calculated as the difference between soluble total N and inorganic N.

In addition, effects of the slurry treatments on the availability of N (NO_3-N, NH_4-N) in the soil was estimated using capsules filled with 1 g of ion exchange resin (UNIBEST Ag Manager™; Unibest International, USA). Three capsules were buried at a depth of 3 cm in each plot on June 25. One capsule was then collected after 2, 4 and 8 weeks from each plot, rinsed with distilled water and stored at 4 °C until processed. Their ion content was extracted using 50 ml of 2 M HCl in the laboratory of Unibest International LLC.

2.1.7. Sampling of soil organisms

To analyze the effects of slurry treatments on the abundance of nematodes (representing the microfauna) and enchytraeids (representing the mesofauna), ten randomly placed soil cores (depth 6 cm, diameter 3 cm) were collected from each manure-treated plot on August 19 (8 weeks after spreading of the slurries). Five cores were taken from the slurry bands and five cores from the area between them. The soil cores were pooled to attain one sample per plot. Nematodes were extracted from ca. 40 g and enchytraeids from ca. 200 g of fresh, non-sieved soil using the wet funnel methods of Sohlenius (1979) and O'Connor (1955), respectively. The number of nematodes was counted, their trophic groups were identified (Yeates et al., 1993) and to examine the effects of treatments further on the community composition of nematodes, relative proportions of trophic groups were calculated. Enchytraeids were counted and classified into size classes (length 0–2, 2.1–4, 4.1–6, 6.1–8, 8.1–10, 10.1–12 or > 12 mm) and their biomass was calculated according to Abrahamsen (1973). Nematode and enchytraeid abundances are expressed per gramme of soil dry matter. The water content of soil samples was determined by weighing subsamples before and after drying in an oven (105 °C) for 24 h.

2.2. Incubation experiments

Three incubation experiments were performed in the laboratory to explain the observed growth responses in the field experiment (see results). In the incubation experiments, the effects of SA and PL treated slurries on soil 1) N mineralization, 2) microbial respiration activity and 3) phytotoxicity were investigated.

Field soil from the field experiment site and untreated cattle slurry collected while establishing the field experiment and stored frozen thereafter were used in the incubations. The slurry was thawed and divided into three equal portions, one of which was left untreated, one acidified with SA (7.8 kg t^{-1}) and one with PL (99 kg t^{-1}) to reach pH 5.2. The slurries were then left to stand undisturbed at room temperature for one week until establishing the incubation treatments.

The field soil was passed through a 15 mm sieve and thereafter weighed in 150 ml plastic pots to total 100 g (DM) per pot. The experimental treatments consisted of 1) untreated soil, 2) soil amended with US, 3) soil amended with slurry + SA and 4) soil amended with slurry + PL. The slurry application rate was chosen to imitate approximately that used in the field study, i.e. 9.0 g US, 9.07 g slurry + SA and 9.89 g slurry + PL was added per pot. Each treatment was conducted in three replicates in each separate incubation set-up described in detail below.

The incubations were carried out in a completely randomized design in a constant temperature chamber at 20 °C. Soil moisture was kept between 30 and 50% from field capacity by adding deionized water according to weight loss approximately twice a week.

2.2.1. Nitrogen mineralization

In the N mineralization set-up, three pots from each treatment were removed and frozen for later analysis after 0, 4, 20, 48 and 64 days from establishing the experiment. Finally, inorganic N was extracted from the entire soil content in each pot (100 g DM) with 250 ml of 1 M KCl and the NH_4-N and NO_3-N concentrations of the extracts analyzed with a Skalar San^{++} autoanalyzer. The mineralization test followed ISO 14238:2013 except for the amount of substrate added and the soil:solution ratio in the KCl extraction, which are specified above.

2.2.2. Phytotoxicity

In the set-up exploring phytotoxicity, three pots from each treatment were removed to a greenhouse to be sown with 30 seeds of watercress (*Nasturtium officinale*) after 0, 14 and 28 days from establishing the experiment. After sowing, the soil surface was smoothly leveled and the pots were covered with transparent plastic for two days to keep the moisture content constant during the germination. Temperature in the greenhouse was adjusted to 18/16 °C and a 16/8 h light cycle. The pots were irrigated three times a week by weighing the pots and applying tap water until achieving initial pot weight. Germination of seedlings was observed 7 and 14 days after sowing. After 14 days, the seedlings were harvested (0.5 cm from soil surface) and their fresh and dry (2 d, 70 °C) weights were taken.

2.2.3. Microbial respiration activity

In the set-up studying respiration following ISO 16072:2002, each experimental pot was placed in a gas-tight 2 l glass container with an open bottle containing 10 ml of 3.5 M NaOH to trap the emitted carbon dioxide (CO_2). The CO_2 traps were renewed after 1, 3, 7, 11, 15, 21, 31 and 50 days from establishing the experiment. The amount of CO_2 produced from each pot was measured via titration of the NaOH solutions with HCl and calculated as the difference between the mean HCl consumption of control traps placed in empty containers and the traps of the treatments. In this experiment, soilless treatments containing 9 g of the slurries, each in three replicates, were also included.

2.3. Statistical analyses

The homogeneity of variances and normal distribution of model residuals were tested using Levene's test and Shapiro-Wilk test, respectively. The effects of slurry treatments on crop properties (yield, N content, root biomass), the abundance and biomass of enchytraeids, the abundance and relative proportions of nematode trophic groups and the nitrogen (NO_3-N, NH_4-N, organic-N) concentrations (response variables) in the soil at the start and end of the field study were tested by multivariate ANOVA using the slurry treatment as a fixed factor. To explain spatial variation within the experimental field the effect of replicate block was included in all models as a random factor.

ANOVA model with randomized blocks for response variable:

$$Response_{ij} = int + Slurry_i + Block_j + e_{ij}$$

where int = intercept Slurry$_i$ = fixed effect of slurry treatment i (i = SA, PL or UN) Block$_j$ = random effect of block j (j = 1, 2, … or 4) e_{ij} = random error of treatment i in block j.

Random terms Block$_j$ and e_{ij} are assumed to be independently and normally distributed with expected value zero and variances σ^2_{Block} and σ^2_e.

Effects of treatments on NH_3 volatilization and resin nutrient capture were tested using ANOVA for repeated measurements. As there was an interaction between sampling time and treatment effect, each sampling time was analyzed separately using one-way ANOVA.

The effects of the slurry treatments on the amounts of NO_3-N, NH_4-N and total KCl-extractable inorganic N (NO_3-N + NH_4-N) in the soil during laboratory incubation was analyzed using multivariate ANOVA by comparing the US, PL and SA. As N incubation analyses were done from different pots at each sampling time (destructive sampling), each time was analyzed separately. In contrast, the effect of slurry treatments on soil CO_2 production (microbial respiration activity) was analyzed using the same pots several times and was thus tested using ANOVA models for repeated measures where the repeated measures comprised samplings. As there were interactions between the time and treatment, each sampling time was analyzed separately using multivariate ANOVA. Germination assay of *N. officinale* seedlings comprised three pot series that were analyzed separately using ANOVA models for repeated measures where the repeated measures comprised time of germination observation (7 and 14 days).

ANOVA model with randomized blocks and repeated measures for response variable Response:

$$Response_{ijk} = int + Slurry_i + Block_j + (Slurry * Block)_{ij} + Time_k + (Slurry * Time)_{ik} + e_{ijk}$$

where int = intercept Slurry$_i$ = fixed effect of slurry treatment i (i = SA, PL or UN) Block$_j$ = random effect of block j (j = 1, 2, … or 4) (Slurry * Block)$_{ij}$ = random interaction effect of treatment i and block j Time$_k$ = fixed effect of time k (k = 7 or 14 d) (Slurry * Time)$_{ik}$ = fixed interaction effect of treatment i and time k e_{ijk} = random error of treatment i in block j at time k.

Random terms Block$_j$, (Slurry * Block)$_{ij}$ and e_{ijk} are assumed to be independently and normally distributed with expected value zero and variances σ^2_{Block}, $\sigma^2_{Slurry*Block}$ and σ^2_e.

Once statistically significant treatment effects appeared in ANOVA models, the statistical significances of differences between treatments were interpreted using a Tukey's post hoc test. All statistical analyses were carried out using the SPSS statistical package (IBM Corp, 2016).

3. Results

3.1. Field experiment

3.1.1. Ammonia volatilization

Air temperature ranged from 11.6 °C in the night-time to 25.6 °C in the daytime, with the mean daily temperature of 20.0 °C on June 17–19 in 2019. During measurements daily wind speed averaged between 1.0 and 2.6 m s^{-1} and precipitation of 1.1 mm occurred between June 17 and 18 (Supplementary Fig. 3). The uppermost soil layers were dry, while volumetric water content increased with depth, ranging from 18 to 21%, 23 to 25%, 30 to 32% and 34 to 37% at the depths of 0–5, 5–10, 10–15 and 15–20 cm, respectively.

The NH_3 volatilization rate from the soils treated with US was on average 3.4 kg NH_3-N ha^{-1} h^{-1} during the first two measurement periods on the day of slurry application. SA- and PL-acidification reduced the NH_3 volatilization significantly to a very low level (< 0.04 kg NH_3-N ha^{-1} h^{-1}) (Fig. 2). On days 1 and 2 after slurry applications, the NH_3 volatilization rate from the US plots declined to an average of 1.0 and 0.3 kg NH_3-N ha^{-1} h^{-1}, respectively, while the NH_3 losses from acidified slurries slightly increased but still remained low (an average of 0.09 kg NH_3-N ha^{-1} h^{-1}). Difference between US and both acidification (SA and PL) treatments was significant in all five samplings (Times 1–4: $P < 0.01$, Time 5: $P = 0.032$). In contrast, no statistically significant differences in NH_3 volatilization existed between PL and SA treated plots (each Time: $P = 0.893$–1.000) (Fig. 2).

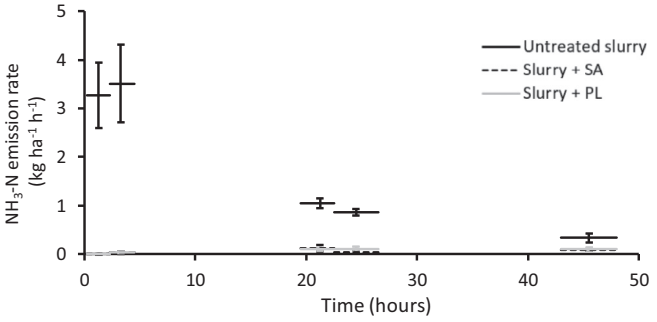


Fig. 2. Average ammonia-N emission rate (kg NH_3-N ha^{-1} h^{-1}) during five series of measurement periods after application of untreated cattle slurry and cattle slurries acidified with sulfuric acid (slurry + SA) and pyrolysis liquid (slurry + PL). Mean and standard deviation (n = 4) are presented for each measurement period.

In the plots treated with US, the average cumulative emission during the five measurement periods amounted to 24 kg NH_3-N ha^{-1}, making up 39% of the initially applied water-extractable NH_4-N. The data from the separate measurement periods were also extrapolated over the whole 49 h period after slurry application by assuming that emissions follow a diurnal pattern and the emission during the night is negligible (Häni et al., 2016), and by deriving the emission value for the intervals between the measurements as an average of two consecutive measurements. According to this conservative estimate, the total cumulative losses in the US may have risen up to 34 kg NH_3-N ha^{-1}, being about 55% of the applied water extractable NH_4-N. The respective estimate for the plots treated with SA and PL acidified slurry was only about 2 kg NH_3-N ha^{-1}, which totaled only about 3% of the applied NH_4-N.

3.1.2. Plant dry matter and nitrogen yields

At the first harvest, for which all plots were similarly fertilized with NK compound fertilizer, the DM and N yields ranged from 4932 to 5341 kg ha^{-1} and 73 to 79 kg ha^{-1}, respectively, with no differences between plots reserved for the different treatments ($P > 0.05$). At the second harvest, for which the slurry additions were applied, acidification with SA resulted in the highest DM and N yields (3161 kg DM ha^{-1},

Table 3
F and *P* statistics of one-way ANOVA of the effects of block and slurry treatment [untreated slurry (US), pyrolysis liquid acidified slurry (PL) and sulfuric acid treated slurry (SA)] on grass DM and N yield (above ground), root biomass and root N yield, supplied with a Tukey's HSD test of the statistical significance of differences among plots and slurry treatments. Statistically significant ($P < 0.05$) effects are marked in bold.

			df	F	*P*	Tukey HSD
Harvest 2	Grass DM	Block	3	0.662	0.598	–
		Treatment	2	16.39	**0.001**	**US = PL < SA**
	Grass N yield	Block	3	0.331	0.803	–
		Treatment	2	27.77	**0.000**	**US = PL < SA**
Harvest 3	Grass DM	Block	3	1.927	0.227	–
		Treatment	2	9.198	**0.015**	**US = PL < SA**
	Grass N yield	Block	3	1.676	0.270	
		Treatment	2	6.078	**0.360**	**US = PL < SA**
	Root DM	Block	3	0.728	0.563	–
		Treatment	2	2.422	0.144	–
	Root N yield	Block	3	0.099	0.958	–
		Treatment	2	4.564	0.051	–

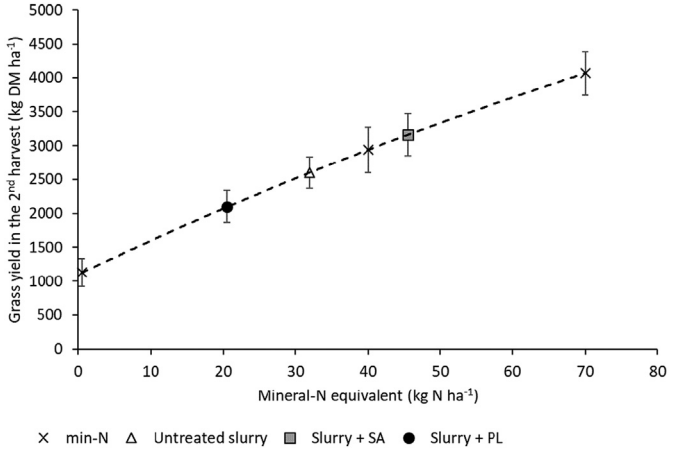


Fig. 3. The yield response curve for the second harvest of ley to increasing mineral N additions. The mineral N equivalents of untreated slurry (N application rate 164 kg tot. N ha^{-1}, 62 kg water-extractable NH_4-N ha^{-1}), slurry acidified with sulfuric acid (SA; 164 kg tot. N ha^{-1}, 68 kg water-extractable NH_4-N ha^{-1}) and slurry acidified with pyrolysis liquid (PL; 144 kg tot. N ha^{-1}, 58 kg water-extractable NH_4-N ha^{-1}) are obtained from the curve according to the yields associated with the treatments.

40 kg N ha^{-1}) among the slurry treatments, differing significantly from the PL acidified and US treatments (Table 3). Acidification with PL produced the lowest yield (2101 kg DM ha^{-1}, 24 kg N ha^{-1}) of the three slurry treatments but the difference of 500 kg ha^{-1} to the US (2603 kg DM ha^{-1}, 27 kg N ha^{-1}) was not statistically significant even if on the practical level it was large.

If calculated from the yield response curve of the grass ley to increasing mineral fertilizer N additions, 32.0, 45.6 kg and 20.5 N_{min} would have been needed to produce similar yields to the US, slurry + SA and slurry + PL, respectively (Fig. 3). Consequently, 19, 28 and 14% of the total N and 52, 67 and 35% of the water-extractable NH_4-N in the untreated, SA and PL slurries, respectively, can be considered to have been equivalent to N_{min}. Apparent N recovery, determined as the proportion of total N added that was recovered in the harvested ley biomass after subtracting the N attained in non-N-fertilized plants, was 47% in the mineral N treatments (40 N_{min}, 70 N_{min}) and 10, 18 and 9% in the US, slurry + SA and slurry + PL treatments, respectively. For water-extractable NH_4-N, the corresponding values for the slurry treatments were 26, 43 and 22%.

In the third harvest grown without additional fertilization, the DM yields and N-uptakes remained low. The SA-acidified slurry treatment produced the largest residual DM yield (400 kg ha^{-1}) and N-uptake (7.2 kg ha^{-1}) in comparison with the US (248 kg DM ha^{-1}, 4.5 kg N ha^{-1}) and PL (252 kg DM ha^{-1}, 4.4 kg N ha^{-1}) treatments (Table 3).

Although the slurry treatments had no statistically significant effect on the plant root DM (ca. 5300–6600 kg ha^{-1}) or root N yield (42–54 kg ha^{-1}) (Table 3), the SA-acidified slurry produced both highest root biomass and root N yield (Supplementary Fig. 4).

3.1.3. Soil mineral N availability

In spring 2019, the experimental field contained on average 0.3 kg NO_3-N ha^{-1}, 5.1 kg NH_4-N ha^{-1} and 27 kg SON ha^{-1} in the 0–20 cm surface layer with no discernible differences between the blocks. After the growing period containing three harvests, the total easily available N (sum of KCl extractable NO_3-N, NH_4-N and SON) content in the upper layer of the soil (0–20 cm) was 33.4, 33.4 and 37.6 kg ha^{-1} in US, SA and PL treated soils with no statistically significant difference between treatments. Neither the soil NH_4-N (5.6–6.1 kg ha^{-1}), NO_3-N (0.30–0.39 kg ha^{-1}) nor SON (27.4–31.2 kg ha^{-1}) differed significantly between the treatments (Statistics: Supplementary Table 1).

In the amount of total inorganic N (NO_3-N + NH_4-N), NO_3-N or NH_4-N captured by resin capsules, no interaction between time and treatment ($P > 0.05$ in each case) was observed. The slurry treatment had a significant effect on the resin-captured total inorganic N (Repeated measures ANOVA; $F = 10.957$, $P = 0.024$) but not on NH_4-N ($F = 4.010$, $P = 0.111$) or NO_3-N ($F = 1.160$, $P = 0.401$) when assessed separately. The amount of NO_3-N, NH_4-N and total inorganic N captured by the capsules after 2, 4 and 8 weeks from the burial in the soil was 19–21%, 52–64% and 41–52%, higher in the US than in the SA and PL treatments, respectively; but due to high variation the difference was statistically significant only with total inorganic N at week 8 (one-way ANOVA; $F = 5.491$, $P = 0.044$) (Fig. 4).

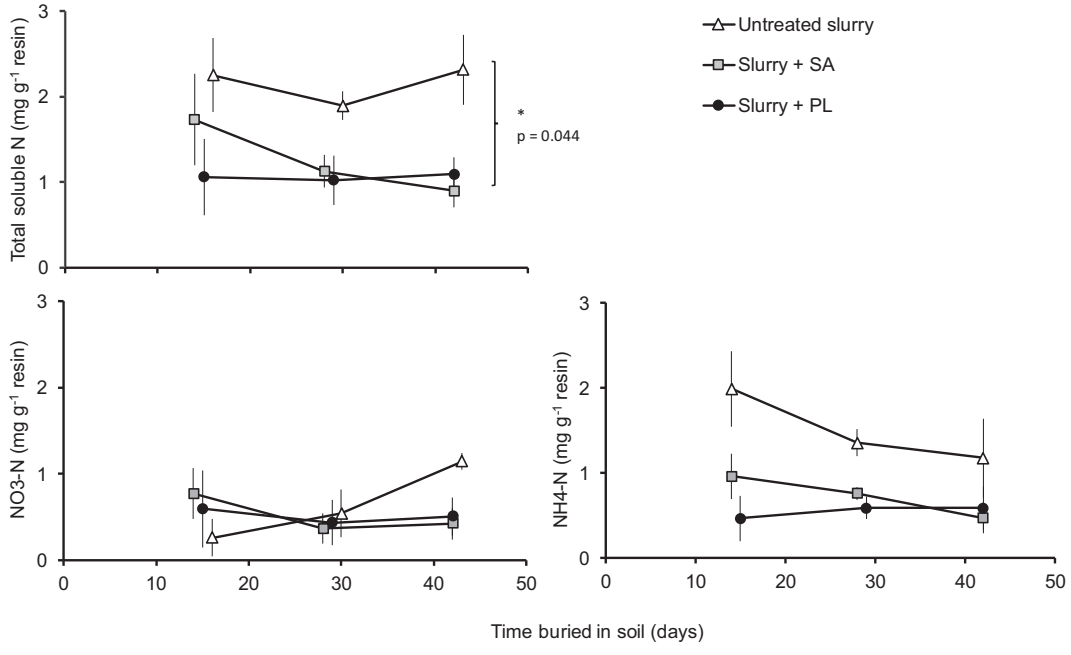


Fig. 4. Amount of inorganic N (mean ± s.e., n = 4) captured by ion exchange resin capsules in 14, 28 and 56 days after burying the capsules in the soil applied with untreated slurry, slurry acidified with sulfuric acid (SA) or pyrolysis liquid (PL). Statistically significant differences ($P < 0.05$) are marked with *.

Table 4
F and *P* statistics of multivariate ANOVA of the effects of block and slurry treatment on abundance and biomass of enchytraeids, total abundance of nematodes, abundance of various trophic groups of nematodes and their relative proportions, supplied with a Tukey's HSD test of the statistical significance of differences among block and slurry treatments. Statistically significant ($P < 0.05$) effects are marked in bold.

		df	F	*P*	Tukey HSD
Enchytraeid abundance	Block	3	3.516	0.770	–
	Treatment	2	2.599	0.143	–
Enchytraeid biomass	Block	3	0.484	0.704	–
	Treatment	2	0.398	0.686	–
Nematode abundance	Block	3	2.162	0.181	–
	Treatment	2	1.956	0.211	–
Total abundance of herbivores	Block	3	0.558	0.657	–
	Treatment	2	1.520	0.270	–
Total abundance of fungivores	Block	3	4.593	**0.038**	**Block 4 < 3, 2, 1**
	Treatment	2	0.369	0.702	–
Total abundance of bacterivores	Block	3	1.347	0.326	–
	Treatment	2	0.797	0.480	–
Total abundance of omnivores	Block	3	1.943	0.201	–
	Treatment	2	0.235	0.795	–
Relative abundance of herbivores	Block	3	1.376	0.318	–
	Treatment	2	0.156	0.850	–
Relative abundance of fungivores	Block	3	0.706	0.575	–
	Treatment	2	0.520	0.611	–
Relative abundance of bacterivores	Block	3	0.340	0.798	–
	Treatment	2	0.078	0.925	–
Relative abundance of omnivores	Block	3	1.200	0.370	–
	Treatment	2	0.079	0.925	–

3.1.4. Effects on soil organisms

Enchytraeid biomass was on average smallest and abundance of enchytraeids highest in SA treated plots, but neither the differences in the number or biomass differed statistically significantly between treatments (Table 4, Supplementary Fig. 5).

Mean abundances of nematodes were not affected by block or slurry treatment (Table 4). Neither treatment effect in the total abundances (Fig. 5) or relative abundances (Supplementary Fig. 6) of bacterivore, fungivore, herbivore, omnivore or predator nematodes were found (Table 4) ($P > 0.05$ in each case). Relative abundances of bacterivore, fungivore, herbivore, omnivore and predator nematodes were 48–55%, 33–40%, 10–14%, 0.3–2% and 0%, respectively (Supplementary Fig. 6).

3.2. Incubation experiments

3.2.1. Nitrogen mineralization

Directly after soil application, the amount of KCl-extractable inorganic N (NH_4-N + NO_3-N, ca. 130 mg N kg^{-1}), extracted from soils amended with untreated slurry was slightly lower than the corresponding amount (ca. 135 mg kg^{-1}) acquired from soils that had received the slurry amended one week before the application with SA or PL (Fig. 6, Table 5). During the first 4 days of incubation, the KCl-extractable N had decreased in all the treatments but remained at higher level in the slurry + SA amended soil than in the US or PL treatments. Thereafter, (21, 50 and 64 days) the extracted N in the US and SA treatments showed a slightly increasing trend ending at nearly the initial level such that the SA treatment yielded somewhat more KCl-extractable inorganic N throughout the study. By contrast, in the slurry + PL treatment, after the drop in the beginning, the KCl-extractable N remained relatively constant and at a significantly lower level than in the US and SA.

In all the treatments, the KCl-extractable inorganic N was first recovered almost completely as NH_4^+, but in the course of the incubation the proportion of NO_3^- quickly increased (Fig. 6). At the end of the incubation, nearly all the recovered N was obtained as NO_3^-. However, in the slurry + PL treatment, the nitrification was somewhat retarded, which was evidenced as significantly highest NH_4-N yield at the 21-day sampling and significantly lowest NO_3-N yield already at day 4.

3.2.2. Phytotoxicity

When *N. officinale* seeds were sown in the soil surface just after the mixing of slurry in the soil (Day 0), SA was shown to reduce the plant germination 7 and 14 days later by 61 and 41%, respectively, whereas PL inhibited seed germination entirely (Fig. 7a). When seeds were sown after 14 days incubation period, a phytotoxic effect was not shown in any treatment (Fig. 7b). After 21 days incubation, PL pots showed slight decrease in plant germination at 7 days after sowing but not after 14 days (Fig. 7c).

3.2.3. Microbial respiration activity — CO_2 production

Soils not amended with slurry emitted CO_2 at a constant rate of 0.05 mg CO_2 g^{-1} dry soil d^{-1} throughout the study period (data not shown), whereas the slurry-amended soils exhibited a clear CO_2 emission peak at the beginning of the incubation period (Fig. 8a, Table 6).

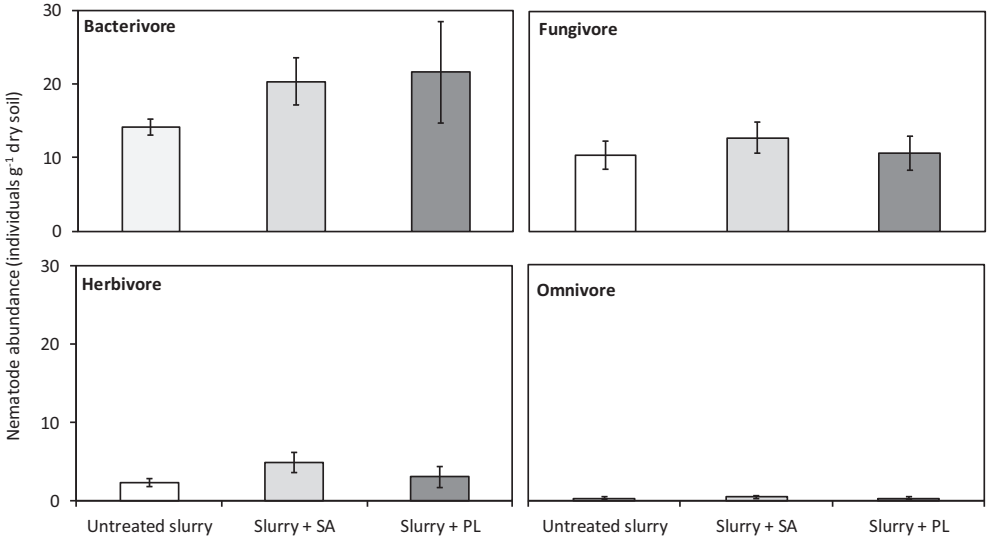


Fig. 5. Abundance of nematode trophic groups (mean + s.e., n = 4) in the soils applied with untreated or variously acidified manure slurry (45/49 t ha^{-1}). SA = sulfuric acid, PL = pyrolysis liquid. Statistically significant differences were not established (see Table 4).

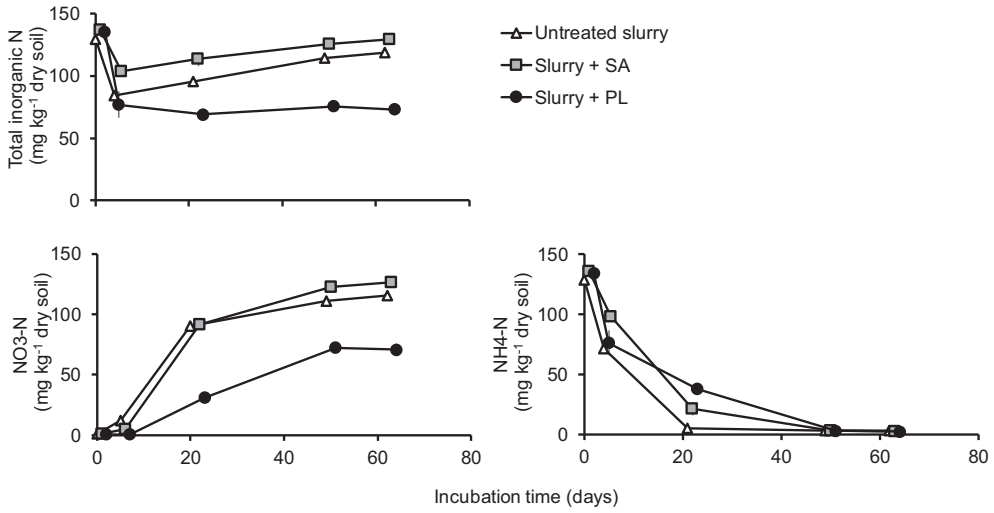


Fig. 6. Amount of inorganic N (mean ± s.d., n = 3) in the soil after 0, 4, 21, 50 and 64 days from the application of untreated slurry, slurry acidified with sulfuric acid (SA) or pyrolysis liquid (PL). For statistics see Table 5.

During the first days after slurry application, the CO_2 emission from soil with slurry + SA was somewhat lower than from soil having received untreated slurry, but thereafter the respiration in the two treatments was practically consistent. In the soil + PL, the lag phase was somewhat longer and the belated CO_2 peak clearly stronger than in the other two slurry treated soils. When incubated without soil, the CO_2 emission from the untreated slurry followed a similar pattern as in the soil mixture (Fig. 8b). However, in the SA acidified slurry, a small peak in the respiration was observed only at the end of the study period, whereas in the PL amended slurry, the CO_2 emission remained at a low level throughout the study.

4. Discussion

Based on our earlier knowledge of SA usability in slurry acidification, PL chemical properties and laboratory scale indications of PL performance in acidification, we proposed four hypotheses which were partly confirmed. (1) As we expected, both SA and PL reduced NH_3 emission almost totally on the day of slurry spreading. However, in contrast to our assumptions (2) the plant biomass and N yield was significantly lower in the PL compared with SA plots. Thirdly, (3) PL induced no harmful effects on soil enchytraeids and nematodes, and (4) PL was rapidly consumed by soil microbes as indicated by increased microbial activity. Taken together, our results suggest that PL performs well as an acidifying agent but due to it containing various potentially harmful organic compounds, a withholding period between soil application and contact with crop seems necessary. Therefore, PL may not be preferred in perennial cropping systems. Furthermore, its effects on soil N dynamics warrant further research.

Table 5
F and *P* statistics of multivariate ANOVA of the effects of slurry treatment [untreated slurry (US), pyrolysis liquid acidified slurry (PL) and sulfuric acid treated slurry (SA)] on NH_4-N, NO_3-N and their sum, in 0, 4, 21, 50 and 64 days after mixing in the soil. A Tukey's HSD shows the statistical significance of differences among slurry treatments. Statistically significant ($P < 0.05$) effects are marked in bold.

	Time	df	F	*P*	Tukey HSD
NH_4-N	0	2	10.31	**0.011**	**US < PL = SA**
	4	2	16.42	**0.004**	**US = PL < SA**
	21	2	58.17	**0.000**	**US < SA < PL**
	50	2	0.231	0.801	–
	64	2	1.430	0.310	–
NO_3-N	0	2	2.951	0.128	–
	4	2	180.1	**0.000**	**PL < SA < US**
	21	2	301.8	**0.000**	**PL < SA = US**
	50	2	250.3	**0.000**	**PL < US < SA**
	64	2	283.5	**0.000**	**PL < US < SA**
Tot N	0	2	9.995	**0.012**	**US < PL = SA**
	4	2	15.88	**0.004**	**US = PL < SA**
	21	2	284.4	**0.000**	**PL < US < SA**
	50	2	301.9	**0.000**	**PL < US < SA**
	64	2	299.4	**0.000**	**PL < US < SA**

4.1. Ammonia volatilization

As we hypothesized, the acidification with both SA and PL prevented NH_3 emission almost completely on the day of slurry spreading. This was congruent with previous studies reviewed by Fangueiro et al. (2015), where the lowering of cattle and pig slurry pH resulted in significant reduction of NH_3 emission rates. In the present study, the differently treated slurries were similar in their relative proportion of water-extractable NH_4-N to total N. In addition to the lower pH, however, the acidified slurries differed from the US in having lower DM contents. Ammonia volatilization from surface-applied slurry has been shown to decline with increased infiltration, being related to the slurry DM content and particle-size distribution of the soil (Sommer et al., 2006). Higher aqueous N infiltration into soil among the acidified slurries might thus have contributed to the decreased NH_3 emissions.

Ammonia volatilization is controlled by prevailing weather conditions, such as temperature, wind speed and air humidity (e.g. Ferm et al., 1999; Hafner et al., 2018, 2019; Rubæk et al., 1996; Sommer and Hutchings, 2001; Sommer et al., 1991). In the present study, warm and dry conditions promoting NH_3 emission prevailed during and after field application of the cattle slurries. Although band spreading, trailing hose/shoe and injection application techniques retard and/or reduce NH_3 losses as compared with broadcast spreading (Häni et al., 2016; Malgeryd, 1998; Mattila and Joki-Tokola, 2003; Pfluke et al., 2011), high NH_3 emission rates were recorded from the US plots on the day of banded application. The emission rate diminished substantially with time such that on the second day it was reduced by about 70% from the level obtained on the first day. The greater part, even up to 90% of the total NH_3 losses, have been shown to occur within the first 12–24 h of slurry applications (e.g. Häni et al., 2016; Pain et al., 1989; Pfluke et al., 2011), emphasizing the importance of effectiveness

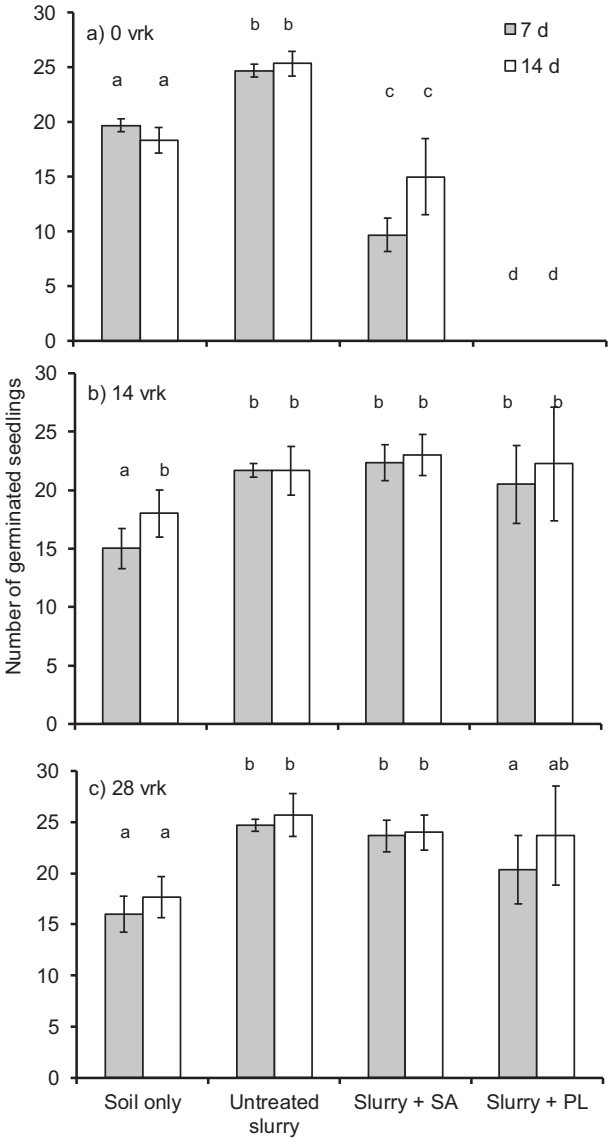


Fig. 7. Number of germinated *N. officinale* seedlings (mean s.e., n = 3) in the pots comprising soil only or mixed with untreated slurry or slurry acidified with sulfuric acid (SA) or pyrolysis liquid (PL). Statistical differences ($P < 0.05$) between bars are marked with different letters.

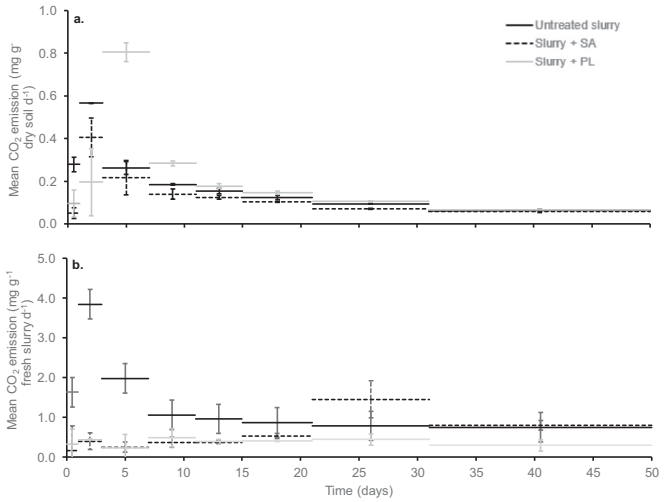


Fig. 8. CO_2 emission per day (mean ± s.d., n = 3) from the (a) soil after the application of untreated slurry (US) or slurry acidified with sulfuric acid (SA) or pyrolysis liquid (PL) and (b) corresponding CO_2 emissions from the differently treated slurries incubated without soil. For statistics see Table 6.

of measures in reducing NH_3 volatilization immediately following slurry spreading.

The total NH_3-N emissions calculated over the whole monitoring period should be treated as approximates due to the assumptions related to extrapolations over non-measured periods (see Section 3.1.1). In this respect, the applied JTI method is weaker than e.g., the online wind tunnel system (Pedersen et al., 2020). The average cumulative NH_3-N emissions of 39–55% of the applied water-extractable NH_4-N (24 kg ha^{-1} during the measurement periods and 34 kg ha^{-1} when extrapolated over 49 h following slurry application) obtained in the current study were somewhat higher than the corresponding value in Mattila and Joki-Tokola (2003) using the same methodology. They reported that on average 31% of the ammoniacal N of cattle slurries was volatilized after band application on to grass ley in mineral and organic soils in southwestern and northern Finland. In the present study, due to a slight rain event after slurry applications, some moisture was retained in the surface-banded slurries to the next day. This might have a promoting influence on NH_3 volatilization, as formation of natural surface crust of field-applied slurry along with drying have been associated with the decrease in the rate of NH_3 loss (Sommer et al., 1991). Moreover, the application rate of band-applied slurries was relatively high in the present study. Higher rate of manure applications has contributed to the higher total NH_3-N losses, being previously associated with the surface broadcast and band treatments (e.g. Pfluke et al., 2011). Overall, our results demonstrate that a marked portion of N can be volatilized from band-applied cattle slurry reducing its fertilizer value substantially. However, volatilization can be effectively reduced by lowering cattle slurry pH using various acids.

Table 6
F and *P* statistics of repeated and one-way ANOVA of the effects of slurry treatment as such [untreated slurry (US), pyrolysis liquid acidified slurry (PL) and sulfuric acid treated slurry (SA)] and mixed with soil on slurry and soil CO_2 production 1–50 days after mixing in the soil. A Tukey's HSD test shows the statistical significance of differences among slurry treatments. Statistically significant ($P < 0.05$) effects are marked in bold.

Treatments		Time (d)	df	F	*P*	Tukey HSD
Soil + slurry	One-way ANOVA	1	2	21.36	**0.002**	**US > PL = SA**
		3	2	9.460	**0.014**	**US > PL, US > SA, PL = SA**
		7	2	103.1	**0.000**	**US = SA < PL**
		11	2	47.17	**0.000**	**US = SA < PL**
		15	2	18.50	**0.003**	**US > SA, PL > SA, US = PL**
		21	2	61.00	**0.000**	**PL > US > SA**
		31	2	48.50	**0.000**	**US = PL > SA**
		50	2	4.200	0.072	–
	Repeated ANOVA	Time × Treatment	14	52.36	**0.000**	
		Time	7	138.0	**0.010**	
Slurry	One-way ANOVA	1	2	10.93	**0.010**	**US > PL = SA**
		3	2	00.93	**0.000**	**US > PL = SA**
		7	2	58.44	**0.000**	**US > PL = SA**
		11	2	15.19	**0.004**	**US > PL = SA**
		15	2	151.2	**0.000**	**US > PL = SA**
		21	2	95.02	**0.000**	**US > PL = SA**
		31	2	9.940	**0.012**	**US = SA, US = PL, SA > PL**
		50	2	15.06	**0.005**	**US = SA > PL**
	Repeated ANOVA	Time × Treatment	14	12.18	**0.000**	
		Time	7	64.15	**0.000**	

4.2. Yield and N recovery

In N limiting conditions, decline in the gaseous losses of N can be expected to lead to an increase in yield due to higher amount of N available for plant uptake. In the current field experiment, a clear yield response to increasing N additions was obtained. Consistently, in the SA acidification treatment, the assumption of higher dry mass production and N uptake in comparison with untreated slurry impoverished by loss of NH_3 was realized. Calculated from the total N contents of the slurry at the time of spreading, roughly 10 percentage points higher mineral N equivalent and apparent N recovery were attained in the SA than US treatment. Sørensen and Eriksen (2009) reported an acidification induced increase of 25 percentage points in slurry mineral N equivalent, whereas Frost et al. (1990) obtained a mean increase of as high as 55 percentage points in the inorganic N equivalence value for slurry NH_4-N due to acidification. However, yield responses to acidification have been inconsistent and the efficiency of the treatment regarding yield in general likely depends on the extent of NH_3 losses (weather conditions during spreading) and the soil properties (e.g. Pain et al., 1989; Seidel et al., 2017). In addition, the effects may be difficult to reliably detect in field conditions due to the small magnitude of preserved N against large variation in manure composition and background soil fertility (Webb et al., 2010; Soinne et al., 2020).

Contrary to our initial hypothesis, an equal growth effect to that observed with SA was not attained in the PL treatment despite similar NH_3 emission reduction by the both acidification agents. As far as S fertilization, Hahtonen and Saarela (1995) investigated the effect of S application rates (3–68 kg ha^{-1}) on grass DM yields at six experimental sites in central and northern Finland. They found that grass growth response to supplementary S was small, inconsistent and statistically insignificant when soil S status ranged from 9 to 30 mg S l^{-1}. In the present study, the mineral N treatment of 70 kg ha^{-1} produced the highest DM yield in the second harvest, receiving 12 kg S ha^{-1} along with NPK fertilizers. Moreover, the PL treatment produced the lower DM yield than US treatment, with equal S application rates, and therefore the results indicate that the difference in DM yields between SA and PL treatments was not explained by the effects of S fertilization. Based on the DM and N yields and consequent mineral N equivalent and apparent N recovery in the slurry + PL treatment, the preserved N was not efficiently utilized by the crop, rather the visual appearance in color and strength of the plant stand having received the PL treated slurry was weaker and the mean harvested yield lower than in the treatment fertilized with US though the yield difference did not become statistically significant. According to slurry N analysis at the time of spreading, approximately 10 kg ha^{-1} less NH_4-N was applied in the PL treatment in comparison with the SA. The N application rate in the PL was even slightly lower than in the US having been exposed to NH_3 emission during the storage period. The exact cause of this discrepancy is not known because the dilution related to high volume of PL addition was compensated for by higher application rate. It is possible that some organic compounds in the PL could have bound NH_4-N to the non-exchangeable form (Nõmmik, 1970). However, it is unlikely that the slurry bound N would not have been released in the Kjeldahl digestion of total N analysis, which also evidenced a similar difference. A more probable explanation is that NH_4-N from the N rich liquid fraction of the slurry was lost due to spontaneous foaming, which occurred in both acidification treatments, but which was more severe in the PL, leading to some flow of slurry over the edges of the container. However, the lower N application rate alone did not account for the weak yield performance of the PL treatment but the low N_{min} equivalence indicated that either restriction in the N uptake by the plants or N availability in the soil occurred. PL containing phytotoxic substances could also have contributed to the lower yields (see Section 4.3).

The amounts of easily available soil N were observed in the field throughout the growth by resin capsules and in addition, before and after the slurry applications by conventional soil sampling. However, these measures were not sensitive enough to reveal any differences between the treatments. Therefore, the soil N dynamics were investigated in more detail in laboratory conditions. In soils amended with US and slurry + SA, the incubation experiment showed an initial net immobilization of mineral N into organic N followed by gradual mineralization, which is a common pattern for animal manures due to microbial N immobilization resulting from introduction of high amounts of available carbon (Azeez and Van Averbeke, 2010; Burger and Venterea, 2008). In the PL treatment, in contrast, the mineralization phase was not observed but the soil inorganic N concentration remained constantly at a lower level than in the soils amended with US or slurry with SA. In previous studies, application of acidified slurry has been found to potentially decrease soil microbial activity in the short-term and consequently decrease N immobilization (Fangueiro et al., 2009, 2013, 2016).

4.3. Ecotoxicological implications

In the field, the possible toxic impacts of the treatments were examined through observing soil nematodes and enchytraeids, which are key organisms in controlling nutrient mineralization (Bardgett, 2005; Ingham et al., 1985; Sulkava et al., 1996). The abundance of enchytraeids and the abundance and proportions of trophic groups of nematodes promptly respond to disturbances in soil (Mikola et al., 2001, 2009). Thus, these groups have been suggested as suitable bioindicators for soil health and quality (Pulleman et al., 2012). Changes in the abundance of bacterial- and fungal-feeding nematodes can be used as surrogates of changes in bacterial and fungal growth (Christensen et al., 1992; Sohlenius, 1990). In the present study, an exceptionally dry growing season reduced abundance of enchytraeids while abundances of more dry tolerant nematodes were high (McSorley, 2003). As we expected, neither the abundance of enchytraeids nor of nematodes or the proportions of the trophic groups of nematodes differed between the treatments. There is limited earlier evidence of PL or SA effects on soil organisms. Recently, Koç et al. (2020) studied the effects of various pesticides and pyrolysis liquid (wood vinegar) applied to the soil surface on soil nematodes. They reported that PL doses and their frequencies could affect the numbers of nematode groups. However, as no parallel effects were found, they suggested further studies for resolving the bio-pesticide potential of PL on nematodes. Furfural, typically found in PL (Fagernas et al., 2012), has previously been used as a nematicide (Hensley and Burger, 2006). The doses of PL (600–2400 l ha^{-1}) used in the study by Koç et al. (2020) were far below those associated with the soil in our experiment (4068 l ha^{-1}). However, the result indicating no differences in the abundance or relative proportions of nematode trophic groups or the number of enchytraeids between the untreated and acidified slurries in the two months after the spreading of slurries, suggests that the effects of slurry acidification with PL or SA on soil degraders can be considered negligible. However, our experiment lasted only one growing season and acidified slurries were applied only once. Thus, the effects of regular applications of PL or SA acidified slurry should be further investigated.

The risk presented by SA for the environment is due to hydronium ions (pH effect) and therefore depends on the buffering capacity of the receiving ecosystem (Cedre, 2006). In the environment, SA is rapidly transformed to hydrogen ions and sulfate ions (ECHA, 2020a) and predicted no effect concentration in the soil ($PNEC_{soil}$) for SA is not defined (ECHA, 2020a). In our study, the pH of slurries was reduced to 5.1–5.3 in both acidification treatments (SA and PL). Consequently, the receiving field soil was supposed to acidify similarly in both treatments. As SA had no negative effect on grass dry matter, CO_2 production or *N. officinale* seeds, the decreases in those variables after PL slurry application are most probably for reasons other than soil acidification. Of the PL containing acids (Table 2) only the concentration of acetic acid slightly exceeded the $PNEC_{soil}$ value (ECHA, 2020b: 470 mg kg^{-1})

after the PL slurry application. Furthermore, in our experiment the toxicity of acids on soil micro-organisms is supposed to be markedly reduced when SA and PL were mixed into slurry one week before soil application. Thus, neutralization, i.e. salt formation and settle down of equilibrium, started already in slurry. Consequently, direct comparison with the $PNEC_{soil}$ values is no longer meaningful. These observations together suggest that the reason for observed changes in CO_2 production, retarded nitrification, phytotoxicity on *N. officinale* seeds and reduced grass yield is not soil acidification.

After the application of PL acidified slurry the concentration of catechol might also reach phytotoxic values in the soil. Hulzebos et al. (1993) reported that catechol significantly inhibited seed germination of lettuce (*Lactuca sativa*) on 0.4 mM (0.04 g l^{-1}) nutrient solution. However, effects on seed germination were not found when catechol was mixed in the soil (>1000 mg kg^{-1}) (Hulzebos et al., 1993). In addition, only one reliable study is available concerning the toxicity of catechol on soil organisms, expressing the lowest observed effect value (LOEC, 42 d) of 500 mg kg^{-1} for compost worm (*Eisenia foetida*) growth (ECHA, 2020c). In our study, the highest catechol concentration in the topsoil after the amendment of PL acidified slurry was 13 mg kg^{-1}. Thus, catechol is not most likely behind the observed germination delay. Also concentrations of phenols, furfural, PAHs and other determined compounds (Table 2) were below the toxic limit values (ECHA, 2020d; Reinikainen, 2007). Further research is required to explain the mechanisms behind reduced yield production in the field, delayed microbial activity in the incubation experiment and reduced *N. officinale* seed germination directly after the PL slurry application. One plausible but complex explanation could be the combined effect of various PL containing organic compounds from which only the main compounds were analyzed in this study. For example, pyrolysis liquids (wood vinegar form *Litchi chinensis*) was previously reported to have antibacterial activity (Yang et al., 2016) against several bacterial strains, but on the other hand, organic acids present in pyrolysis liquids are easily degraded by soil microbes.

4.4. Microbial degradation of PL

Increased CO_2 production at day 10, following the incorporation of slurry in soil, suggests that organic compounds present in PL are quickly decomposed by soil micro-organisms. During the 50 days incubation, PL increased the total CO_2 flux ca. 25 and 40% from the soils compared with US and SA treatments (respectively). This is a typical reaction when added resources are rapidly consumed by soil microbes (Hagner et al., 2010; Meli et al., 2003). The difference in respiration to US may be slightly underestimated because release of inorganic carbonate-derived CO_2 after soil incorporation may have contributed to the total CO_2 flux in the US treatment (Fangueiro et al., 2013). In the acidified slurries this fraction was emitted already during the acidification process. Rapid microbial decomposition is also supported by the cress test, as improved germination of *N. officinale* seeds at day 14 showed that the phytotoxic effect of PL had disappeared. The increased microbial activity was noted only when the PL acidified slurry was mixed with the soil, showing that soil functional responses to acidified slurry application depend on chemical characteristics of the acidified agent and soil.

When PL or SA were mixed in the slurry (without soil) their CO_2 production remained significantly lower in comparison with US. Previously, Ottosen et al. (2009) showed that oxygen consumption rate, methanogenesis and sulfate reduction were reduced by more than 98% in the stored SA acidified pig slurry compared with untreated slurry. According to Ottosen et al. (2009) the reduced pH as such cannot explain the greatly compromised microbial activity in the acidified slurry as both the sulfate reduction and methanogenesis occur at low pH values. In their study, reduced microbial activity was explained by the high concentration of protonated volatile fatty acids (VFAs) in the acidified slurry, arresting microbial metabolism. Also Habtewold et al. (2018) found a negative effect of manure acidification on the activities of methanogens. However, the diversity and relative proportions of bacterial communities were not altered with slurry acidification (to pH 5.9) (Habtewold et al., 2018). In our study, the mixing of slurries with soil reversed the microbial inhibiting effect of acidification. This might be due to the dilution of the VFA concentrations of PL and SA below the microbe-inhibiting concentration and further – in PL treatment – the ability of microbes originating from both the slurry and inherently inhabiting the soil to degrade PL-containing organic compounds.

4.5. Practical observations

The volumes of acids needed to reach the target slurry pH were calculated based on a preliminary small-scale test. As for the PL, the target pH was easily achievable without additional readjustment of slurry pH, whereas with SA the adjustment was more challenging. From an industrial safety point of view, working with PL was also more effortless as compared with SA. Moreover, the inherent unpleasant odor of cattle slurry was partly compensated for by a strong tar-like odor of PL. According to visual observations, in exception to the other slurry treatments, the PL treated slurry bands attracted no flies. In addition, the PL treated slurry bands tended to form a harder crust upon drying that might have had a restrictive influence on the N availability in the slurry. However, a large amount of foaming diminished the manageability of acidified slurries during the day of acidification and the following day. Considering the large-scale adoption of proposed usage of PL in manure acidification, the current availability of PL's in markets is limited. Although thermochemical conversion technologies allow condensation of liquid fraction, all volatiles are often incinerated as there are no current market demand for the liquids. Finding new ways to utilize PL would likely improve the environmental and economic performance of the pyrolysis process. Moreover, use of PL e.g. in manure acidification enhance sustainable use of biomass as higher proportion of initial feedstock can be converted to bio-based value-added products.

5. Conclusions

To conclude, our results suggest that PL is as effective as SA in cattle slurry acidification, minimizing NH_3 emissions by up to 99%. The required application rate of PL is, however, many times that of SA, which is disadvantageous in volume-limited conditions. In addition, PL may increase the tendency of slurry foaming in comparison with SA. The organic compounds contained in the PL are readily consumed by soil microbes such that phytotoxic impacts seem transitory and no long-term adverse effects on soil key organisms are expected. However, a withholding period of one to two weeks between soil application and contact with the crop seems necessary and therefore PL may not be feasible in perennial cropping systems. In addition, the boost in soil microbial activity caused by the easily degradable organic compounds in the PL may lead to N immobilization and consequent reduction in the slurry N fertilizer value.

Funding

The experimental part of the study was conducted under the project "Use of pyrolysis products in securing the fertilizer value of liquid manures" (PYSTI) funded by the Ministry of the Environment of Finland from the programme to promote the recycling of nutrients and improve the status of the Archipelago Sea.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Marleena Hagner: Conceptualization, Methodology, Resources, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. **Mari Räty:** Conceptualization, Methodology, Resources, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation. **Johanna Nikama:** Conceptualization, Methodology, Resources, Investigation, Formal analysis, Writing – review & editing, Validation. **Kimmo Rasa:** Conceptualization, Methodology, Resources, Investigation, Formal analysis, Writing – review & editing, Validation. **Sari Peltonen:** Resources, Investigation, Formal analysis, Writing – review & editing, Validation. **Jouko Vepsäläinen:** Resources, Investigation, Formal analysis, Writing – review & editing, Validation. **Riikka Keskinen:** Funding acquisition, Supervision, Conceptualization, Methodology, Resources, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We thank Luke's technical staff in Jokioinen and Maaninka for their help in the setting up and maintaining the field experiment and for collaboration in laboratory analyses. Interest and helpful comments by the project steering group are gratefully acknowledged. Juha Hyvönen is thanked for his advice with statistical models.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147139.

References

Abrahamsen, G., 1973. Biomass and body-surface area of population of enchytraeidae and lumbricidae (Oligochaeta) in Norweigian forest soils. Pedobiologia 13, 28–39.
Azeez, J.O., Van Averbeke, W., 2010. Nitrogen mineralization potential of three animal manures applied on a sandy clay loam soil. Bioresour. Technol. 101, 5645–5651.
Bardgett, R.D., 2005. The Biology of Soils. Oxford University Press.
Beusen, A.H.W., Bouwman, A.F., Heuberger, P.S.C., Van Drecht, G., Van Der Hoekd, K.W., 2008. Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. Atmos. Environ. 42, 6067–6077.
Burger, M., Venterea, R.T., 2008. Nitrogen immobilization and mineralization kinetics of cattle, hog, and turkey manure applied to soil. Soil Sci. Soc. Am. J. 72, 1570–1579.
Campisi, T., Samorì, C., Torri, C., Barbera, G., Foschini, A., Kiwan, A., Galletti, P., Tagliavini, E., Pasteris, A., 2016. Chemical and ecotoxicological properties of three bio-oils from pyrolysis of biomasses. Ecotoxicol. Environ. Saf. 132, 87–93.
Cedre, 2006. Sulphuric acid. Chemical response guide. 64 pp. https://wwz.cedre.fr/en/content/download/2777/140102/file/Extract-sulphuric-acid.pdf Visited 20.8.2020.
Christensen, H., Griffiths, B., Christensen, S., 1992. Bacterial incorporation of tritiated thymidine and populations of bacteriophagous fauna in the rhizosphere of wheat. Soil Biol. Biochem. 24, 703–709.
Diebold, J.P., 1997. A Review of the Toxicity of Biomass Pyrolysis Liquids Formed at Low Temperatures. National Renewable Energy Laboratory, Golden, CO NREL/TP-430-22739, 35 pp.
ECHA, 2020a. Sulphuric Acid https://echa.europa.eu/registration-dossier/−/registered-dossier/16122/6/1 Visited 20.8.2020.
ECHA, 2020b. Acetic Acid https://echa.europa.eu/fi/registration-dossier/−/registered-dossier/15549/6/1 Visited 20.8.2020.
ECHA, 2020c. Pyrocatechol https://echa.europa.eu/cs/registration-dossier/−/registered-dossier/10516/6/4/2 Visited 20.8.2020.
ECHA, 2020d. Search on chemicals. https://echa.europa.eu/information-on-chemicals Visited 20.8.2020.
EN 13652, 2001. Soil improvers and growing media — extraction of water soluble nutrients and elements. Technical Committee CEN/TC 223 "Soil Improvers and Growing Media".
Fagernas, L., Kuoppala, E., Tiilikkala, K., Oasmaa, A., 2012. Chemical composition of birch wood slow pyrolysis products. Energy Fuels 26, 1275–1283.
Fangueiro, D., Ribeiro, H., Vasconcelos, E., Coutinho, J., Cabral, F., 2009. Treatment by acidification followed by solid-liquid separation affects slurry and slurry fractions composition and their potential of N mineralization. Bioresour. Technol. 100, 4914–4917.
Fangueiro, D., Surgy, S., Coutinho, J., Vasconcelos, E., 2013. Impact of cattle slurry acidification on carbon and nitrogen dynamics during storage and after soil incorporation. J. Plant Nutr. Soil Sci. 176, 540–550.
Fangueiro, D., Hjorth, M., Gioelli, F., 2015. Acidification of animal slurry — a review. J. Environ. Manag. 149, 46–56. https://doi.org/10.1016/j.jenvman.2014.10.001.
Fangueiro, D., Surgy, S., Fraga, I., Girão Monteiro, F., Cabral, F., Coutinho, J., 2016. Acidification of animal slurry affects the nitrogen dynamics after soil application. Geoderma 281, 30–38.
Ferm, M., Kasimir-Klemedtsson, Å., Weslien, P., Klemedtsson, L., 1999. Emission of NH_3 and N_2O after spreading of pig slurry by broadcasting or band spreading. Soil Use Manage. 15, 27–33.
Frost, J.P., Stevens, R.J., Laughlin, R.J., 1990. Effect of separation and acidification of cattle slurry on ammonia volatilization and on the efficiency of slurry nitrogen for herbage production. J. Agr. Sci.-Cambridge 115, 49–56.
Giannadaki, D., Giannakis, E., Pozzer, A., Lelieveld, J., 2018. Estimating health and economic benefits of reductions in air pollution from agriculture. Sci. Total Environ. 622–623, 1304–1316.
Habtewold, J., Gordon, R., Sokolov, V., Vander Zaag, A., Wagner-Riddle, C., Dunfield, K., 2018. Reduction in methane emissions from acidified dairy slurry is related to inhibition of Methanosarcina species. Front. Microbiol. 9, 2806. https://doi.org/10.3389/fmicb.2018.02806.
Hafner, S.D., Pacholski, A., Bittman, S., Burchill, W., Bussink, W., Chantigny, M., Carozzi, M., Génermont, S., Häni, C., Hansen, M.N., Huijsmans, J., Hunt, D., Kupper, T., Lanigan, G., Loubet, B., Misselbrook, T., Meisinger, J.J., Neftel, A., Nyord, T., Pedersen, S.V., Sintermann, J., Thompson, R.B., Vermeulen, B., Vestergaard, A.V., Voylokov, P., Williams, J.R., Sommer, S.G., 2018. The ALFAM2 database on ammonia emission from field-applied manure: description and illustrative analysis. Agric. For. Meteorol. 258, 66–79.
Hafner, S.D., Pacholski, A., Bittman, S., Carozzi, M., Chantigny, M., Génermont, S., Häni, C., Hansen, M.N., Huijsmans, J., Kupper, T., Misselbrook, T., Neftel, A., Nyord, T., Sommer, S.G., 2019. A flexible semi-empirical model for estimating ammonia volatilization from field-applied slurry. Atmos. Environ. 199, 474–484.
Hagner, M., 2013. Potential of Slow Pyrolysis Products Birch Tar Oil, Wood Vinegar and Biochar in Sustainable Plant Protection – Pesticidal Effects, Soil Improvement and Environmental Risks Academic Dissertation, Reports from the Department of Environmental Sciences, Lahti, Unigrafia. Helsinki, Finland. ISBN 978-952-10-9169-8 (pdf).
Hagner, M., Pasanen, T., Lindqvist, B., Lindqvist, I., Tiilikkala, K., Penttinen, O.-P., Setälä, H., 2010. Effects of birch tar oils on soil organisms and plants. Agr. Food Sci. 19, 13–23.
Hagner, M., Tiilikkala, K., Lindqvist, I., Niemelä, K., Wikberg, H., Källi, A., Rasa, K., 2020. Performance of liquids from slow pyrolysis and hydrothermal carbonization in plant protection. Waste Biom. Val. 11, 1005–1016. https://doi.org/10.1007/s12649-018-00545-1.
Hahtonen, M., Saarela, I., 1995. The effects of sulphur application on yield, sulphur content and N/S-ratio of grasses for silage at six sites in Finland. Acta Agric. Scand. Sect. B Soil Plant Sci. 45, 104–111.
Häni, C., Sintermann, J., Kupper, T., Jocher, M., Neftel, A., 2016. Ammonia emission after slurry application to grassland in Switzerland. Atmos. Environ. 125, 92–99.
Hartung, J., Phillips, V.R., 1994. Control of gaseous emissions from livestock buildings and manure stores. J. Agric. Eng. Res. 57, 173–189.
Hensley, J., Burger, G., 2006. Nematicidal properties of furfural and the development for nematode control in various crops for the United States markets. J. Nematol. 38, 274.
Hulzebos, E.M., Dirven-Van Breemen, E.M., van Dis, W.A., Herbold, H.A., Hoekstra, J.A., Baerselman, R., van Gestel, C.A.M., Adema, D.M.M., Henzen, L., 1993. Phytotoxicity studies with *Lactuca sativa* in soil and nutrient solution. Environ. Toxicol. Chem. 12, 1079–1094. https://doi.org/10.1002/etc.5620120614.
Hyrkäs, M., Virkajärvi, P., 2014. Säilörehunurmen rikkilannoitus 2013. In: Kanninen, J. (Ed.), Lannoitus- ja kasvinsuojelukokeiden tuloksia 2013. Maa- ja elintarviketalouden tutkimuskeskus (MTT), Yara Suomi Oy, pp. 35–36 (in Finnish).
IBM Corp, 2016. IBM SPSS Statistics for Windows, Version 24.0. IBM Corp., Armonk, NY.
Ingham, R.E., Trofymow, J.A., Ingham, E.R., Coleman, D.C., 1985. Interactions of bacteria, fungi and their nematode grazers: effects on nutrient cycling and plant growth. Ecol. Monogr. 55, 119–140.
IUSS Working Group WRB, 2015. World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.
Kai, P., Pedersen, P., Jensen, J.E., Hansen, M.N., Sommer, S.G., 2008. A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. Eur. J. Agron. 28, 148–154.
Kanter, D.R., 2018. Nitrogen pollution: a key building block for addressing climate change. Clim. Change 147, 11–21.
Kemppainen, E., 1989. Nutrient content and fertilizer value of livestock manure with special reference to cow manure. Ann. Agric. Fenn. 28, 163–284.
Keskinen, R., Hyväluoma, J., Wikberg, H., Källi, A., Salo, T., Rasa, K., 2018. Possibilities of using liquids from slow pyrolysis and hydrothermal carbonization in acidification of animal slurry. Waste Biom. Valor 9, 1429–1433.
Koç, İ., Yıldız, Ş., Yardım, E.C., 2020. Effect of some pesticides and wood vinegar on soil nematodes in a wheat agro-ecosystem. KSU J. Agric. Nat. 23, 621–633. https://doi.org/10.18016/ksutarimdoga.vi.570180.
Libra, J.A., Ro, K.S., Kammann, C., Funke, A., Berge, N.D., Neubauer, Y., Titirici, M.-M., Fühner, C., Bens, O., Kern, J., Emmerich, K.-H., 2011. Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. Biofuels 2, 71–106.
Malgeryd, J., 1998. Technical measures to reduce ammonia losses after spreading of animal manure. Nutr. Cycl. Agroecosyst. 51, 51–57.

Mattila, P., 2006. Ammonia Emissions From Pig and Cattle Slurry in the Field and Utilization of Slurry Nitrogen in Crop Production. Doctoral Dissertation. Agrifood Research Reports 87. MTT Agrifood Research Finland 136 p.

Mattila, P.K., Joki-Tokola, E., 2003. Effect of treatment and application technique of cattle slurry on its utilization by ley: I. Slurry properties and ammonia volatilization. Nutr. Cycl. Agroecosyst. 65, 221–230.

McCubbin, D.R., Apelberg, B.J., Roe, S., Divita Jr., F., 2002. Livestock ammonia management and particulate-related health benefits. Environ. Sci. Technol. 36, 1141–1146.

McSorley, R., 2003. Adaptations of nematodes to environmental extremes. Fla. Entomol. 86, 138–142. https://doi.org/10.1653/0015-4040(2003)086[0138:AONTEE]2.0.CO;2.

Meli, S.M., Badalucco, L., English, L.C., Hopkins, D.W., 2003. Respiratory responses of soil micro-organisms to simple and complex organic substances. Biol. Fert. Soils 37, 96–101.

Mikola, J., Setälä, H., 1998. Productivity and trophic-level biomasses in a microbial-based soil food web. Oikos 82, 158–168.

Mikola, J., Yeates, G.W., Barker, G.M., Wardle, D.A., Bonner, K.I., 2001. Effects of defoliation intensity on soil food-web properties in an experimental grassland community. Oikos 92, 333–343.

Mikola, J., Setälä, H., Virkajärvi, P., Saarijärvi, K., Ilmarinen, K., Voigt, W., Vestberg, M., 2009. Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. Ecol. Monogr. 79, 221–224. https://doi.org/10.1890/08-1846.1.

Ndegwa, P.M., Hristov, A.N., Arogo, J., Sheffield, R.E., 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. Biosyst. Eng. 100, 453–469.

Nõmmik, H., 1970. Non-exchangeable binding of ammonium and amino nitrogen by Norway spruce raw humus. Plant Soil 33, 581–595.

O'Connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. Nature 175, 815–816.

Ottosen, L.D.M., Poulsen, H.V., Nielsen, D.A., Finster, K., Nielsen, L.P., Revsbech, N.P., 2009. Observations on microbial activity in acidified pig slurry. Bios. Eng. 102, 291–297.

Pain, B.F., Phillips, V.R., Clarkson, C.R., Klarenbeek, J.V., 1989. Loss of nitrogen through ammonia volatilisation during and following the application of pig or cattle slurry to grassland. J. Sci. Food Agric. 47, 1–12.

Pedersen, J.M., Feilberg, A., Kamp, J.N., Hafner, S., Nyord, T., 2020. Ammonia emission measurement with an online wind tunnel system for evaluation of manure application techniques. Atmos. Environ. 230, 117562.

Pfluke, P.D., Jokela, W.E., Bosworth, S.C., 2011. Ammonia volatilization from surface-banded and broadcast application of liquid dairy manure on grass forage. J. Environ. Qual. 40, 374–382.

Pirinen, P., Simola, H., Aalto, J., Kaukoranta, J.-P., Karlsson, P., Ruuhela, R., 2012. Climatological statistics of Finland 1981–2010. Reports 2012:1. Finnish Meteorological Institute, Helsinki, Finland 83 p.

Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Pérès, G., Rutgers, M., 2012. Soil biodiversity, biological indicators and soil ecosystem services — an overview of European approaches. Curr. Opin. Environ. Sust. 4, 529–538.

Reinikainen, J., 2007. Maaperän kynnys ja ohjearvojen määritysperusteet. Suomen ympäristö 23. Suomen ympäristökeskus, Helsinki 168 p.

Rubæk, G.H., Henriksen, K., Petersen, J., Rasmussen, B., Sommer, S.G., 1996. Effects of application technique and anaerobic digestion on gaseous nitrogen loss from animal slurry applied to ryegrass (*Lolium perenne*). J. Agric. Sci. 126, 481–492.

Seidel, A., Pacholski, A., Nyord, T., Vestergaard, A., Pahlmann, I., Herrman, A., Kage, H., 2017. Effects of acidification and injection of pasture applied cattle slurry on ammonia losses, N_2O emissions and crop N uptake. Agric. Ecosyst. Environ. 247, 23–32.

Smucker, A.J.M., McBurney, S.L., Srivastava, A.K., 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74, 500–503.

Sohlenius, B., 1990. Influence of cropping system and nitrogen input on soil fauna and microorganisms in a Swedish arable soil. Biol. Fertil. Soils 9, 168–173.

Sohlenius, B.A., 1979. Carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. Holarctic Ecol. 2, 30–40.

Soinne, H., Keskinen, R., Räty, M., Kanerva, S., Turtola, E., Kaseva, J., Nuutinen, V., Simojoki, A., Salo, T., 2020. Soil organic carbon and clay content as deciding factors for net nitrogen mineralization and cereal yields in boreal mineral soils. Eur. J. Soil Sci., 1–16 https://doi.org/10.1111/ejss.13003.

Sommer, S.G., Hutchings, N.J., 2001. Ammonia emission from field applied manure and its reduction — invited paper. Eur. J. Agron. 15, 1–15.

Sommer, S.G., Olesen, J.E., Christensen, B.T., 1991. Effects of temperature, wind speed and air humidity on ammonia volatilization from surface applied cattle slurry. J. Agric. Sci. 117, 91–100.

Sommer, S.G., Jensen, L.S., Clausen, S.B., Søgaard, H.T., 2006. Ammonia volatilization from surface-applied livestock slurry as affected by slurry composition and slurry infiltration depth. J. Agric. Sci. 144, 229–235.

Sørensen, P., Eriksen, J., 2009. Effects of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. Agric. Ecosyst. Environ. 131, 240–246.

Sulkava, P., Huhta, V., Laakso, J., 1996. Impact of soil faunal structure on decomposition and N-mineralisation in relation to temperature and moisture in forest soil. Pedobiologia 40, 505–513.

Svensson, L., 1994. A new dynamic chamber technique for measuring ammonia emissions from land-spread manure and fertilizers. Acta Agr. Scand. B-S P 44, 35–46.

Tang, Y.S., Flechard, C.R., Dämmgen, U., Vidic, S., Djuricic, V., Mitosinkova, M., Uggerud, H.T., Sanz, M.J., Simmons, I., Dragosits, U., Nemitz, E., Twigg, M., van Dijk, N., Fauvel, Y., Sanz, F., Ferm, M., Perrino, C., Catrambone, M., Leaver, D., Braban, C.F., Cape, J.N., Heal, M.R., Sutton, M.A., 2021. Pan-European rural monitoring network shows dominance of NH_3 gas and NH_4NO_3 aerosol in inorganic atmospheric pollution load. Atmos. Chem. Phys. 21, 875–914.

Vuorinen, J., Mäkitie, O., 1955. The method of soil testing in use in Finland. Agrogeol. Publ. 63, 1–44.

Webb, J., Pain, B., Bittman, S., Morgan, J., 2010. The impacts of manure application methods of ammonia, nitrous oxide and on crop response — a review. Agric. Ecosyst. Environ. 137, 39–46.

Yang, J.Y., Yang, C.H., Liang, M.T., Gao, Z.J., Wu, Y.W., Chuang, L.Y., 2016. Chemical composition, antioxidant, and antibacterial activity of wood vinegar from *Litchi chinensis*. Molecules 21, 1150.

Yeates, G.W., Bongers, T., RGM, De Goede, Freckman, D.W., Georgieva, S.S., 1993. Feeding-habits in soil nematode families and genera: an outline for soil ecologists. J. Nematol. 25, 315–331.