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Short communication

Assessing refrigerating and freezing effects on the biological/chemical composition of two livestock manures

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ABSTRACT

Assessing storage impacts on manure properties is relevant to research associated with nutrient-use efficiency and greenhouse gas (GHG) emissions. We examined the impact of cold storage on physicochemical properties, biochemical methane-emitting potential (BMP) and the composition of microbial communities of beef feedlot manure and poultry broiler litter. Manures were analysed within 2 days of collection and after 2 and 8 weeks in refrigerated (4 °C) or frozen (–20 °C) storage. Compared with fresh manure, stored manures had statistically significant ($p < 0.05$) but comparatively minor (<10%) changes in electrical conductivity, chloride and ammonium concentrations. Refrigeration and freezing did not significantly affect ($p > 0.05$) BMP in both manure types. We did not detect ammonium- or nitrite-oxidising bacterial taxa (AOB, NOB) using fluorescence in situ hybridisation (FISH). Importantly, the viability of microbes was unchanged by storage. We conclude that storage at –20 °C or 4 °C adequately preserves the investigated traits of the studied manures for research aimed at improving nutrient cycling and reducing GHG emissions.

1. Introduction

Handling manure is a centuries-old issue for livestock farmers (Potter et al., 2010). Recently, a number of factors have sparked increased focus on effective manure management that include (i) tightening of regulations on nutrient leaching (Elofsson, 2013), (ii) increased awareness of the negative environmental impacts of manure gaseous emissions especially nitrous oxide, methane and ammonia (Chadwick et al., 2011; Wu et al., 2013) and (iii) escalating costs of synthetic and mineral fertilisers combined with dwindling and non-renewable natural deposits (Cordell et al., 2009). In light of these factors, avenues are sought to minimise the negative environmental impacts associated with handling manure whilst harnessing the resource potential of manures to full effect (Chadwick et al., 2011; Redding, 2011).

To investigate strategies for optimising manure management, researchers often collect livestock manures for experimentation, but it is generally impractical to perform tests on fresh manures due to the distance to research facilities and timing of analyses. Consequently, manure samples are preserved prior to analysis by freeze- or oven-drying, refrigeration or freezing (Mahimairaja et al., 1990; Van Kessel et al., 1999; Pan et al., 2009). It has been well documented that the composition of manure changes with time during storage in the field (Markewich et al., 2010), while little is known about the effects on preservation methods on biological and physicochemical properties of manures.

Van Kessel et al. (1999) examined how nitrogen (N) and carbon (C) turnover in mixtures of soil and dairy manure is affected by preservation methods. The authors concluded that freeze-drying and oven-drying had considerable impact on manure composition, with a 30% or higher decrease in N concentrations in preserved samples. By contrast, refrigeration and freezing of manure had negligible impact on N and C turnover (Van Kessel et al., 1999). However, Pan et al. (2009) reported that refrigeration of poultry manure drastically altered N composition with a 5-fold decrease and increase in urea–N and ammonium–N, respectively, compared with freezing, freeze-drying or acidification. Preservation methods were ranked in order of least to strongest impact on manure composition with freezing > freeze-drying > acidification > refrigeration (Pan et al., 2009). However, freezing, which did not greatly

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affect manure composition, was still considered potentially problematic for subsequent manure experimentation (Pan et al., 2009).

The contrasting results of previous studies (Van Kessel et al., 1999; Pan et al., 2009) may be attributable to the different manures tested but also demonstrate that impacts of different preservation techniques on manure composition are not well understood. Hence, we aimed to investigate how two commonly used preservation methods (refrigeration and freezing) affect manure samples from poultry and beef industries. Our scope is focused on research pertaining to nutrient use efficiency and greenhouse gas emissions from manures applied to arable soils. Hence, we targeted manures from waste management systems because these are often sought as fertiliser materials.

2. Methods

2.1. Location and sample collection

Five kilograms of manure from a beef feedlot and litter from a poultry shed were used as substrates in this study. The beef feedlot was located in the Darling Downs region (27.3044°S, 151.3583°E), approximately 200 km west of Brisbane, Australia. The feedlot has an average herd size of 17,000 with a stocking density of 15 m^2/livestock unit. Feed materials are variable and include sorghum and barley. Manure, collected to a depth of 10 cm, was obtained from several locations across the feedlot. Manure is scraped out of pens on a 6 month basis so the obtained sample comprised a mixture of fresh and degraded material. The poultry litter sample was collected from a shed on a 30,000-livestock unit meat poultry farm, approximately 50 km west of Brisbane (27.2944°S, 152.2056°E). The shed's stocking rate is 19.5 livestock units/m^2 and litter comprised a combination of bedding (sawdust), manure and feathers. The farm employs the single litter/batch rotation that is common practice in Australian poultry farms with an average period of batch occupation of 8 weeks. Approximately, 5 kg of beef manure and poultry litter was each homogenised using an industrial-scale mixer (HOBART).

2.2. Sample storage

Homogenised materials were split into several batches (1) set-aside for immediate analyses within 1 day of collection being kept on ice during transportation from the farms to the research laboratory, (2) placed in a refrigerator (4 °C) for 2 weeks, (3) stored in a refrigerator for 8 weeks, (4) stored in a freezer (−20 °C) for 2 weeks and (5) stored in a freezer for 8 weeks. The batches were then further split into sub-batches for analyses of physicochemical properties, methane emission potential and composition of the microbial community.

2.3. Physicochemical analyses and biochemical methane potential tests

Moisture content was determined by oven-drying 50 g of the samples overnight at 105 °C and recording weight loss. The pH was measured in 1:5 manure:water extracts. Total Kjeldahl nitrogen (N) and phosphorus (P) TKN and TKP were analysed colourimetrically using the Kjeldahl wet oxidation digestion process (Crowther et al., 1980). Total N and C were quantified (LECO analyser model, make) following Dumas dry combustion principle where samples are combusted at 1050 °C (Buckee, 1994). Organic C was quantified colourimetrically after digestion with H_2SO_4 and $K_2Cr_2O_7$ (Walkley and Black, 1934). Ammonia–N and NO_2–N were analysed following extraction with 2 M KCl (1:10 manure/water ratio) and measured colorimetrically (Bremner and Keeney, 1965). Nitrate–N was determined in 2 M KCl extract followed by steam distillation with MgO, addition of Devarda's alloy and titration using 0.01 M HCl (Bremner and Keeney, 1965). Colwell phosphorus (P) was determined by shaking samples for 16 h end-over-end at a 1:20 ratio with deionised water, adjusted to pH 8.5 with 0.5 M sodium hydrogen carbonate; extracts were then filtered and analysed for ortho-phosphate (Saggar et al., 1999). Sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe) and sulphur (S) were measured by inductively-coupled plasma mass spectrometry following an aqua-regia digest. The procedure is detailed in Crosland et al. (1995). The biochemical methane potential (BMP) tests were conducted by the Advanced Water Management Centre (University of Queensland). Their methods are well-described in other publications (Tait et al., 2009).

2.4. Fluorescence in-situ hybridisation (FISH)

Manure samples (approximately 1 g) were fixed by adding three volumes of 4% paraformaldehyde in 1× phosphate-buffered saline (PBS) and incubating at 4 °C for 3 h, and then washed in PBS and stored in PBS with 50% ethanol at −20 °C. To prepare samples for FISH, bacteria were first separated from the bulk of the auto-fluorescent non-bacterial particles by centrifugation through a Histodenz (equivalent to Nycodenz, Sigma Chemical Co.) density gradient as described by Bertaux et al. (2007). Bacteria were then immobilised on Isopore GTTP membranes (Millipore) and dehydrated in an ethanol series as described by Bertaux et al. (2007). Fluorescence in-situ hybridisation was carried out following the protocol of Bertaux et al. (2007), except that each hybridisation was done in a separate well of a 24-well flat bottom tissue culture plate. We used oligonucleotide probes targeting ammonia oxidising Betaproteobacteria [Nso1225; (Mobarry et al., 1996)] and species in the nitrite oxidising bacterial genus *Nitrospira* [NSR827; (Schramm et al., 1998)] in combination with a probe targeting most bacteria [EUB338; (Amann et al., 1990)]. Following hybridisation and washing, samples were imaged using a Zeiss LSM700 Confocal Microscope.

2.5. Quality assurance

All physicochemical, microbial analyses and biochemical methane potential tests were conducted in triplicate. Blanks with no methanogen-seeded inoculum were run during the methane potential tests.

2.6. Statistical analysis

Repeated measures analysis of variance (ANOVA) was conducted on the manure physicochemical parameters in order to assess significance of changes with storage (4 °C and −20 °C) and time (0, 2 and 8 weeks). The time-series data were taken into account by an analysis of variance of repeated measures (Rowell and Walters, 1976), via the AREPMEASURES procedure of GenStat (2013) which forms an approximate split-plot analysis of variance (split for time). The Greenhouse–Geisser epsilon estimates the degree of temporal autocorrelation and adjusts the probability levels for this. For the methane potential tests, the ultimate biochemical methane potential (B_o) was determined using a gradient search technique with the sum of squared errors as the objective function, and parameter uncertainty estimated from the linear region around the optimum (95% confidence two-tailed *t*-test). The analysis was performed using the AQUASIM software (version 2.1d).