Dairy manure as a potential source of crop nutrients and environmental contaminants

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ABSTRACT

Although animal manure is applied to agricultural fields for its nutrient value, it may also contain potential contaminants. To determine the variability in such contaminants as well as in valuable nutrients, nine uncomposted manure samples from Idaho dairies collected during 2.5 years were analyzed for macro- and micro-nutrients, hormones, phytoestrogens, antibiotics, veterinary drugs, antibiotic resistance genes, and genetic elements involved in the spread of antibiotic resistance. Total N ranged from 6.8 to 30.7 (C:N of 10 to 21), P from 2.4 to 9.0, and K from 10.2 to 47.7 g/kg manure. Zn (103 – 348 mg/kg) was more abundant than Cu (56 – 127 mg/kg) in all samples. Phytoestrogens were the most prevalent contaminants detected, with concentrations fluctuating over time, reflecting animal diets. This is the first study to document the presence of flunixin, a non-steroidal anti-inflammatory drug, in solid stacked manure from regular dairy operations. Monensin was the most frequently detected antibiotic. Progesterones and sulfonamides were regularly detected. We also investigated the relative abundance of several types of plasmids involved in the spread of antibiotic resistance in clinical settings. Plasmids belonging to the IncI, IncP, and IncQ1 incompatibility groups were found in almost all manure samples. IncQ1 plasmids, class 1 integrons, and sulfonamide resistance genes were the most widespread and abundant genetic element surveyed, emphasizing their potential role in the spread of antibiotic resistance. The benefits associated with amending agricultural soils with dairy manure must be carefully weighed against the potential negative consequences of any manure contaminants.

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Introduction

Dairy production will continue to increase in order to supply the growing global demand for protein sources that meet the nutritional requirements of consumers (Chagunda et al., 2016; Flachowsky et al., 2018; Lagrange et al., 2015). As a consequence, manure production will correspondingly increase, resulting in concentrated sources of waste materials that are typically applied to agricultural fields to provide essential plant nutrients and organic matter (Diacono and Montemurro, 2010). Due to its nutritional abundance, regular application of manure has been shown to increase macro- and micronutrient concentrations in degraded soils (Diacono and Montemurro, 2010; Garcia et al., 2017; Larney et al., 2011). Although manure is applied at a rate to match its available nutrients to a crop's requirements, much of the total nitrogen (N) and phosphorus (P) in manure occur in their organic forms, which are not immediately plant-available. Mineralization of organic substrates to bioavailable nitrate (NO_3^--N), ammonium (NH_4^+-N), and phosphate over several years can result in overapplication of total N and P to an agricultural field, especially after repeated applica-

tions. Consequently, leaching of nitrate into groundwater is a threat to human health (Ahada and Suthar, 2018; Basso and Ritchie, 2005; Biddau et al., 2019; Di and Cameron, 2002; Zhou et al., 2016) and excess N and P in surface water cause eutrophication (Luo et al., 2017).

Along with these macronutrients applied with manure to meet crop needs, micronutrients present in the manure can also accumulate in soil (Sheppard and Sanipelli, 2012). Copper (Cu) and zinc (Zn) are food additives used by the dairy industry that concentrate in manure (Griffiths et al., 2007; Wysocka et al., 2019). They are also used as sulfates in footbaths to prevent hoof diseases, and the leftover solution is often disposed with the manure (Downing et al., 2010). Cu and Zn have been shown to accumulate in soil after manure application (Diacono and Montemurro, 2010; Imseng et al., 2019; Li et al., 2019; Sheppard and Sanipelli, 2012), possibly because metal sorption to soil increases when applied with an organic fertilizer (Antoniadis, 2008; Guan et al., 2011; Song et al., 2017).

In addition to well-characterized contaminants, many additives are frequently used in modern intensive animal agriculture. As a consequence, the manure it generates has become a potential source of antibiotics, synthetic and endogenous hormones, growth promoters, and antibiotic resistant bacteria (Albero et al., 2014; Campagnolo et al., 2002; Feng et al., 2016; Meyer et al., 2000; Pruden et al., 2016; Ray et al., 2017). The application of manure containing such emerging contaminants (ECs) provides a direct route for ECs to impact environmental or human health through soil leaching, runoff, and plant uptake (Fahrenfeld et al., 2014; Fisher and Scott, 2008; Marti et al., 2013; Pan and Chu, 2017; Tien et al., 2017). Significant concentrations of antibiotics, antibiotic resistant bacteria, and antibiotic resistance genes (ARGs) have been identified in surveys of livestock manure, agricultural soils receiving land-applied manure, and surface and groundwater in agricultural areas (Campagnolo et al., 2002; Muurinen et al., 2017; Noyes et al., 2016; Thanner et al., 2016; Wichmann et al., 2014; Wolters et al., 2016; Zhu et al., 2013). Dairy cattle pose unique challenges with respect to ECs, because of their constant cycle of pregnancy and lactation, whereby the animals excrete large quantities of endogenous hormones primarily as estrogens and progesterones (Hanselman et al., 2003; Zheng et al., 2008). In addition, a diet of soy and clover can result in high concentrations of plant-derived estrogenic compounds in dairy cow manure (Hoerger et al., 2011; Tucker et al., 2010). These so-called phytoestrogens have been shown to cause significant endocrine disruption in multiple fish species both in vivo and in vitro (DiMaggio et al., 2016; Jarošová et al., 2015; Latonnelle et al., 2002; Nezafatian et al., 2017). Excess hormones and phytoestrogens in surface water severely affect aquatic species that are particularly sensitive to endocrine disrupting compounds (Lange et al., 2012; Schubert et al., 2014).

Antibiotics (and metals) are ECs of unique concern because of their potential to promote the spread of multidrug-resistant bacteria, leading to the loss of drug efficacy (World Health Organization, 2014). Many of the antibiotics distributed for human use - including penicillin, tetracyclines, and sulfonamides - are also approved for use in dairy cows. Sick cattle are administered various antibiotics, up to 80% of which can pass unmetabolized through the animal (Kemper et al., 2008), as verified by frequent detections of antibiotics and antibiotic resistant bacteria or genes in dairy manure (Dungan et al., 2018; Kyselková et al., 2015; Munir and Xagoraraki, 2011; Wichmann et al., 2014). As a result of dairy manure application to agricultural fields, substantial amounts of antibiotics and antibiotic resistant bacteria or their genes can persist in soil for several months (Marti et al., 2013; McKinney et al., 2018; Sandberg and LaPara, 2016). However, whether dairy manure represents a significant route for transfer of antibiotic resistant bacteria or genes to human and animal pathogens has not been determined (Tien et al., 2017).

To assess the benefits and potential threats of land application of manure to environmental and human health, it is imperative to quantify temporal variation of the nutrients and contaminants it contains. We therefore analyzed uncomposted dairy manure produced at commercial dairy operations in Southern Idaho. It is important to note that Idaho is the third largest milk producing state in the U.S. (USDA-ERS, 2019) and nearly all of its ~500 dairies are located in Southern Idaho (Dairy West, 2018), resulting in the accumulation of large quantities of manure in a concentrated geographic region. The objective of this study was to identify and quantify nutrients and ECs in manure during a 2.5-year period. Analyses to determine the presence and potential impacts of ECs included direct measurements and assessment of biomarkers indicating the presence of ARGs.

1. Materials and methods

1.1. Sample collection and storage

Manure was collected prior to composting from Magic Valley Composting (MVC) in Jerome, Idaho, a facility that accepts manure from approximately 40 dairies within 100 km (M. de Haro Martí, personal communication). Manure may be stored for weeks or months at the dairies before arriving at MVC, thus the exact age of each analyzed manure sample was unknown. Samples obtained represent likely scenarios for manure management, ensure a representative and random subsample of dairy manure, and protect individual dairies from liability concerns.

Nine composite samples of uncomposted dairy manure from MVC were obtained between June 2015 and January 2017 following University of Idaho's extension protocol (Moore et al., 2015). Each composite sample was comprised of eight subsamples from one windrow, with four subsamples collected from each side. Subsamples were taken from fresh, unturned windrows of dairy manure. The eight subsamples were mixed thoroughly, and the resultant homogeneous composite sample was transferred to two one-gallon plastic bags. One bag was sent to Soiltest Farm Consultants, Inc. (Moses Lake, WA, USA) for nutritional and metal analysis, and the other bag was shipped overnight on ice to our laboratory and immediately stored at 4 °C. Standardized Test Methods for the Examination of Composting and Compost (TMECC) (Thompson et al., 2002) used for manure analysis are detailed in Appendix A **Table S1**.

1.2. Manure extraction and chemical analysis

Manure was extracted and analyzed for twenty ECs: six hormones, four phytoestrogens, eight antibiotics, and two veterinary drugs (**Table 1**). Extraction methods were adapted from Aga et al. (2005) using acidified acetone (pH = 4.0) and citric acid. Detailed extraction and analysis protocols are provided as Appendix A **Table S2**. Briefly, approximately 1 g dry weight manure was spiked with 0.1 µg 2,3,4–^{13}C$_3$–17β-estradiol and ^{13}C$_6$-phenylsulfadimethoxine to monitor extraction and recovery. The extract was concentrated on an Oasis HLB 3cc solid phase extraction (SPE) cartridge previously conditioned with 3 mL of methanol, 3 mL of water, and 1 mL of citric acid. The cartridge was dried under vacuum for 30 min. Cartridges were stored at −20 °C and eluted within 18 wk. SPE cartridges were eluted with 2 mL of 50:50 methanol:acetone and 2 mL of methanol. SPE extracts were evaporated to 1 mL under nitrogen (N$_2$) gas and analyzed by HPLC-MS-ToF (high performance liquid chromatography- mass spectrometry-time of flight) (Agilent, Santa Clara, CA, USA) on a Kinetex C18 column (Phenomenex, Torrance, CA, USA).

1.3. Detection of mobile genetic elements and ARGs

Total DNA was extracted from manure samples in triplicate using the PowerSoil® DNA isolation kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions, except that approximately 125 mg of manure was used for extraction (weights were recorded for each individual extraction) and eluted in 50 µL of buffer. An extraction control without manure was included in each set of DNA extractions. DNA samples were maintained at −20 °C until analysis.

The quantitative real-time polymerase chain reaction (qPCR) methods used to identify the different targets are described in **Table 2**. All qPCR reactions were performed in a total volume of 10 or 20 µL with 1 or 2 µL of total DNA, respectively, using the PerfeCta® qPCR Tough-Mix 2X (Quanta BioSciences, Beverly, MA, USA) or the Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and a primer/probe according to the published method (**Table 2**). The qPCR assays were performed in duplicate with a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using cycling programs described in the published method (**Table 2**). In order to

Table 1 – Organic compounds quantified in manure extracts.

EC Group	Emerging contaminants
Hormone	17α-estradiol, 17β-estradiol, estrone, estriol, progesterone, 17α-hydroxyprogesterone
Phytoestrogen	enterodiol, formononetin, biochanin A, equol
Antibiotic	sulfamethazine, sulfadimethoxine, sulfamethoxazole, sulfathiazole, tetracycline, oxytetracycline, chlortetracycline, penicillin G
Others	flunixin, monensin

Table 2 – Genetic elements targeted by real-time qPCR in dairy manure samples.

Genetic element	Gene targeted	References	Reaction volume (μL)	DNA used to construct standard[a]
16S rRNA encoding gene	V3-V4 region of 16S rRNA gene	Liu et al., 2012	10	Genomic DNA EC100
Class 1 integrons	Integrase *intI1*	Barraud et al., 2010	20	pB10 [a]
IncP plasmid	*korB*	Jechalke et al., 2013	20	pB10 [a]
IncI1 plasmid	*traI*	Blau et al., 2018	20	R64
IncI2 plasmid	*traI*	Blau et al., 2018	20	pHNSHP45
IncQ1 plasmid	*repB*	This study	20	RSF1010
IncF plasmid	*traI*	Blau et al., 2018	20	F
Sulfonamide resistance gene	*sul1*	Pei et al., 2006	20	pB10
Sulfonamide resistance gene	*sul2*	Pei et al., 2006	20	peH4H

[a] The plasmid pB10 was used as a standard plasmid for class 1 integrons and the IncP plasmids.

avoid qPCR inhibitor effects, total DNA samples were diluted 10-fold. Readily available plasmids were used to construct a standard curve in duplicate in each qPCR run. The limit of quantification (LOQ) of the qPCR assay was determined as the serial dilution of each standard curve with the lowest concentration of standard plasmid DNA at which the standard deviation for technical replicates was low according to the StepOnePlus™ Software v2.3 (Applied Biosystems, Foster City, CA, USA). LOQs are reported per gram of the average weight of dry manure used for DNA extraction and account for DNA elution volume, dilution factor, and DNA volume used for real-time qPCR. Efficiencies of qPCR ranged from 87.4% to 105%. Amplification results were analyzed using StepOnePlus™ Software v2.3 (Applied Biosystems, Foster City, CA, USA).

For all but class 1 integrons and IncP (i.e. IncP-1) plasmid quantifications, standard plasmids were constructed as described in Blau et al. (2018) by amplifying each target from the DNA molecules described in Table 2 with the corresponding primer pairs used for real-time qPCR. PCR reactions were performed in a 50 μL final volume using the following mixture: Master Mix 2X (Thermo Scientific, Waltham, MA, USA), 0.3 μmol/L of each primer (Millipore Sigma, Burlington, MA, USA), and 2 μL of the DNA template. The plasmid pB10 was used as a standard plasmid for class 1 integrons and the IncP plasmids.

The primers and probe used to detect the IncQ1 plasmid were based on phylogenetic relationships of the IncQ plasmid family reported in the publication by Loftie-Eaton and Rawlings (2012). Sequences of the *repB* gene of plasmids of the IncQ1 subgroup were aligned, and conserved nucleotide sequences were used for the design of forward primer qIncQ1-fw (CGARGAAYTATCAGGCAT), reverse primer qIncQ1-rv (GTCTTGCCSYTGGAYTCM), and TaqMan probe qIncQ1-tp (CTTGTC-CTTGCGGTTGGT), resulting in a PCR product of 220 bp. The temperature program used for the IncQ1 qPCR was 10 min at 95 °C followed by 40 cycles of 30 s at 95 °C and 60 s at 60 °C.

1.4. Data analysis

Chromatographic data processing was performed with Analyst QS 1.1 software (Applied Biosystems, Foster City, CA, USA). Compounds were identified by their retention times and the specific product masses resulting from fragmentation. Calibration standards were included with each use of the HPLC-MS-ToF to account for day-to-day variability. All calibration curves had an r^2 value of 0.98 or greater. Limits of detection (LOD) for HPLC-MS-ToF were based on a least square regression of

$3.3(s_y \, b^{-1})$, where s_y equals the residual standard deviation of the calibration curve and b is the slope of the line (Appendix A **Table S2**). The analytical error of HPLC-MS-ToF analysis did not exceeded 10% based on the internal quality control and calibration sample analysis. Compounds positively identified in the HPLC-MS-ToF but below the LOD were recorded as below detection limits (BDL). Observations that were identified as BDL were independently evaluated according to the methods described in the Environmental Protection Agency's Data Quality Assessment (USEPA, 2006), and were ultimately treated as not present in the data analysis.

Manure nutrient and physical data obtained from Soiltest Farm Consultants were analyzed for correlation using the "proc corr" command in SAS 9.4 (SAS Institute Inc., 2013). Copy numbers of target genes are reported per gram of dry manure, and normalized gene copy numbers were calculated by dividing these copy numbers by the copy number of the 16S rRNA encoding gene. The correlation between the log transformation of the relative abundance of genes was tested using Spearman's rank correlation coefficient according to the package ggpubr in R (R Core Team, 2017).

1.5. Data presentation

Samples were collected on June 12, August 18, and October 22 of 2015; March 25, May 6, June 2, July 28, and November 1 of 2016; and January 20 of 2017 (Table 3). To allow for visualization of seasonal trends, the data in the figures are presented in chronological order based on month, regardless of the respective year in which samples were collected.
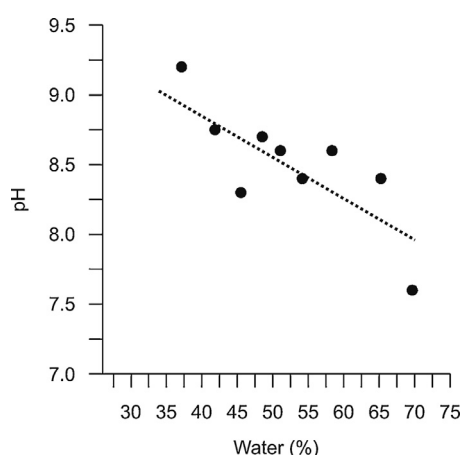
2. Results and discussion

2.1. pH

Manure pH was assessed in this study because of its critical influence on nutrient and metal availability, microbial activity, and behavior of organic compounds. Water content of the manure, which ranged from 37.1% to 69.7% (Table 3), negatively correlated with manure pH (7.6 to 9.2), with drier manure samples having a more basic pH (Fig. 1, $r = -0.80$, $p = 0.0102$). Basic pH values recorded here are consistent with the practice of applying various types of manure to treat acid soils and improve crop productivity (Benke et al., 2010; Butler and Muir, 2006;

Table 3 – Collection dates and selected chemical properties of the manure composite samples.

Date received	Water content (%)	TC (%)	TN (%)	C:N	pH	EC (mmhos/cm)
6/12/15	69.66	21.93	1.02	21.6	7.6	3.05
8/18/15	51.06	25.34	2.41	10.5	8.6	9.17
10/22/15	48.50	23.30	1.59	14.5	8.7	6.51
3/25/16	54.15	15.02	1.17	12.9	8.4	3.95
5/6/16	58.35	12.82	0.68	18.7	8.6	2.90
6/2/16	41.83	10.97	0.91	12.05	8.8	4.46
7/28/16	37.12	32.72	3.07	10.7	9.2	10.73
11/1/16	45.49	19.55	1.89	10.4	8.3	9.29
1/20/17	65.23	12.21	1.22	10.0	8.4	4.71

All values reported on a dry manure weight basis. C:N = carbon to nitrogen ratio; EC = electrical conductivity; TC = total carbon; TN = total nitrogen.

Fig. 1 – Correlation of percent water and pH in dairy manure composite samples.

Butterly et al., 2013; Lupwayi et al., 2014; Whalen et al., 2000). However, long-term studies on the effect of regular manure applications on soil pH have produced inconsistent results due to the complexity of the system (Butterly et al., 2013; Diacono and Montemurro, 2010; Repsiene and Karcauskiene, 2016; Whalen et al., 2000; Zglobicki et al., 2016). Inherent soil properties, climatic and environmental factors, historical land management, and manure composition influence the extent by which manure additions influence soil pH (Eghball, 1999; Gaskell and Smith, 2007; Hutjens, 1998; Ozlu and Kumar, 2018; Repsiene and Karcauskiene, 2016; Vašák et al., 2016).

The cause of the negative correlation between manure water content and pH (Fig. 1) is unknown but may be explained in part by the complex equilibrium among carbonate and N-species in manure, and by microbial activities (Cáceres et al., 2006; García-González et al., 2015; Huang et al., 2004; Husted et al., 1991; Sommer and Husted, 1995; Stevens and Cornforth, 1974; Vanotti et al., 2017; Wen and Brooker, 1995).

Various mechanisms have been proposed to explain manure pH changes due to aeration. Husted et al. (1991) describe it as a balance between the buffering systems of NH_3/NH_4^+ and $CO_2/HCO_3^-/CO_3^{2-}$. Since dissolved CO_2 (i.e., carbonic acid) is far less soluble than NH_3 and very unstable, CO_2 rapidly volatilizes and causes the manure pH to rapidly increase. Alternatively, aeration degrades natural bicarbonate (HCO_3^-) in manure and releases CO_2 and hydroxide ions, the latter causing a significant and rapid pH rise (García-González et al., 2015; Vanotti et al., 2017). Stevens and Cornforth (1974) suggest that less- or non-aerated manure samples contain a greater concentration of dissolved CO_2 (H_2CO_3), which converts to bicarbonate between pHs of 4.3 and 8.3, the typical pH range of fresh dairy manure (McDonald, 2006).

Bicarbonate will then complex with ammonia to produce ammonium bicarbonate, thereby maintaining manure at a near neutral pH (Stevens and Cornforth, 1974). Indeed, most NH_4^+ in swine manure is complexed as ammonium bicarbonate (Zhang et al., 2017). Manure aeration also decreases manure water content by evaporation, thereby increasing the concentration of total ammoniacal N (NH_3 plus NH_4^+) (Huang et al., 2004; Panetta et al., 2005). Concentrations of NH_4^+-N and NO_3^--N observed in our samples reflect trends generally seen in aerobically stored fresh manure; in the first 1–2 weeks, NH_4^+-N increases while NO_3^--N concentrations remain virtually undetectable (Huang et al., 2004). The C:N ratio of manure samples varied from 10 to 21.

The relatively high concentrations of NH_4^+-N in the manure (Fig. 2) may have partially arisen from microbial activities. Alkaline conditions promote mineralization of organic N as illustrated by maximum urease activity in cattle feces occurring at about pH 8.0 (Dai and Karring, 2014). Urea present in cow urine is rapidly hydrolyzed enzymatically by urease produced by fecal microbes, such that within 20 h of mixing cow urine and feces, urea can completely convert to ammonia and carbonic acid (Bibby and Hukins, 1992; Dai and Karring, 2014; Moraes et al., 2017). Given urease's high catalytic activity, pH increases seen in this study were likely not strongly influenced by urea hydrolysis. Yet, we cannot definitively assess the impact of urea mineralization because ammonia volatilization depends on temperature, pH, and storage and handling conditions (Johannesson et al., 2018; Sigurdarson et al., 2018). The greater rise in pH with increased O_2 aeration may also have arisen from increased rate of organic matter degradation (Stevens and Cornforth, 1974; Lambie et al., 2013). Regardless of the underlying mechanism, a range of 1.5 pH units that can potentially be controlled by moisture content has important implications in regulating microbial activity, and thus, chemical transformations, metal solubility, and preservation of ARGs.

2.2. Nutrients

Nutrient analysis of the manure samples, as expressed on a dry weight basis, produced widely variable results (Appendix A **Table S3**). Total N ranged from 6.8 to 30.7, P from 2.4 to 9.0, and K from 10.2 to 47.7 g/kg manure (Table 3 and Fig. 2). The highest potential N:P:K value of the tested manures was equivalent to a fertilizer value of 3:2:6 and the lowest fertilizer value was approximately 1:1:1. The wide range in N:P:K values and the large coefficients of variance (CV) for each of the analyzed nutrients, which ranged from 20% to 212% (Appendix A Table S3), illustrate the high nutrient variability that can be expected in manure. Similar variability has been reported in other manure studies (Jokela et al., 2010; Lorimor et al., 2004). For example, CVs of total N, NH_4^+-N, P, and K were 59%, 81%, 39%, and 74%, respectively, from a survey of uncomposted manure from Vermont dairies (Jokela et al., 2010) and were 50, 60, 43, and 51%, respectively, from our study.

In the present study, the mean total N concentration was 1.6% (dry weight basis) (Supplementary Table S3), which was similar to the 1 to < 3% previously reported for solid dairy manure (Lehrsch et al., 2017; Lentz and Lehrsch, 2018; Manitoba AFRD, 2015). Measured to-
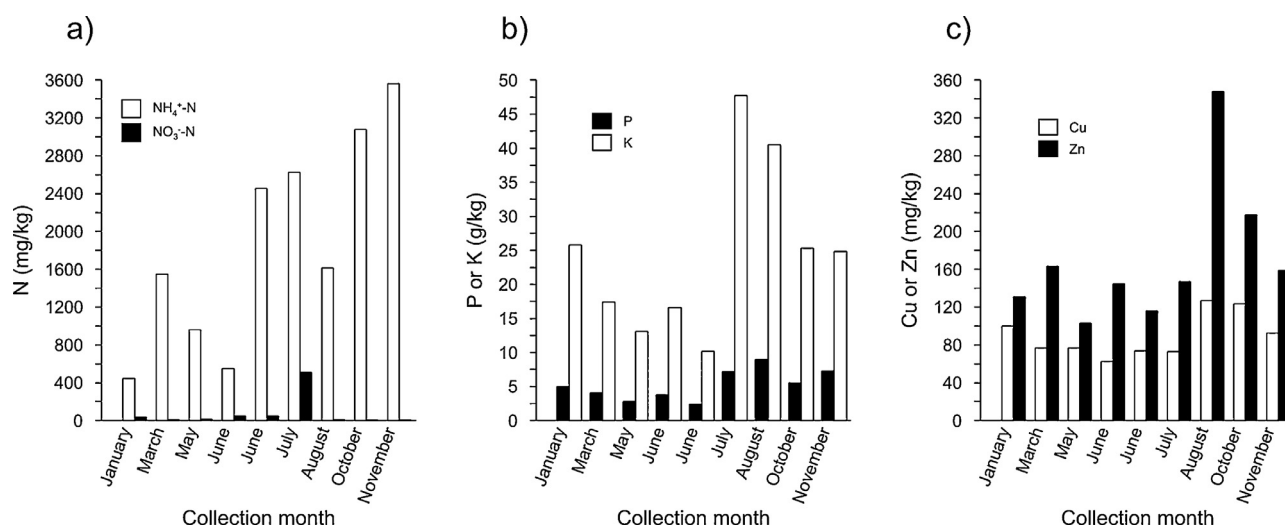
Fig. 2 – Nutrients and metals present in dairy manure composite samples for the respective sampling months. (a) Available ammonium nitrogen (NH$_4^+$-N) and nitrate nitrogen (NO$_3^-$-N); (b) Total phosphorus (P) and potassium (K); (c) Concentrations of copper (Cu) and zinc (Zn).

tal P concentrations (2.4 to 9.0 g/kg) were likewise similar to reported ranges of about 2 to 8 g/kg (Jokela et al., 2010; Kleinman et al., 2009; Lentz and Lehrsch, 2018; Lorimor et al., 2004; Manitoba AFRD, 2015). Measured total K concentrations of 10.2–47.7 g/kg represent a range that is slightly higher than has typically been reported for manure (3 to 37 g/kg) (Jokela et al., 2010; Lehrsch et al., 2014; Lorimor et al., 2004; Manitoba AFRD, 2015). High K concentration in manure is consistent with findings that manure application increases soil K content or runoff concentrations (Domingo-Olivé et al., 2016; Lehrsch et al., 2014; Zglobicki et al., 2016).

The C:N ratio of manure samples in this study ranged from 10:1 to 21:1 (Table 3), very similar to reported dairy manure C:N ratios of 10:1 to 22:1 (Lehrsch et al., 2017; Lehrsch and Kincaid, 2007; Lentz and Lehrsch, 2018; Wang et al., 2014). The calculated median C:N ratio of all collected samples was 12:1. When the C:N ratio of organic residues is approximately 24:1, the rates of microbial N mineralization and immobilization will be equal (Eash et al., 2016). Greater C:N ratios result in a net N immobilization, whereas net N mineralization occurs at C:N ratios less than 24:1. A median C:N ratio of 12:1 indicates that N will be readily available for plant uptake (Qian and Schoenau, 2002; Diacono and Montemurro, 2010).

Manure concentrations of NH$_4^+$-N and NO$_3^-$-N observed in this study reflect trends generally seen in aerobically stored fresh manure (Lehrsch and Kincaid, 2007; Manitoba AFRD, 2015; Martin et al., 2011; Meisinger and Jokela, 2000; Todd et al., 2011). NH$_4^+$-N was the primary inorganic N species detected in the manure samples (500–3500 mg/kg), and was typically present at one or more orders of magnitude higher than nitrate (Fig. 2).

Inorganic N typically constitutes a small proportion of total N in dairy manure (1–25%) (Bhogal et al., 2016; Chadwick et al., 2000; Eghball, 2000; Muñoz et al., 2003; Niu et al., 2017). Despite its small proportion of total N relative to organic N, mobilized inorganic N can become a potential environmental contaminant and threat to human health via erosion (Gangbazo et al., 1995; Lehrsch et al., 2014; Viney et al., 2000), runoff, and leaching (Ahada and Suthar, 2018; Basso and Ritchie, 2005; Biddau et al., 2019; Di and Cameron, 2002; Gallet et al., 2003; Hepperly et al., 2009; Huang et al., 2017; Lentz and Lehrsch, 2018; Zhou et al., 2016). Phosphorus in dairy manure is likewise subject to mobilization, by which it negatively impacts water quality through leaching and surface runoff of particulate and soluble forms (Indiati et al., 1995; Kleinman et al., 2009; Kumaragamage and Akinremi, 2018; Li et al., 2016; Nest et al., 2016; Schelde et al., 2006; Weyers et al., 2017). Excessive N and P loading into surface waters leads to eutrophication, a major environmental problem that disrupts the equilibrium of aquatic ecosystems and results in harmful consequences on ecological and human health (Luo et al., 2017; Yang et al., 2008).

2.3. Metals

Zn (103 to 348 mg/kg) was more abundant than Cu (56 to 127 mg/kg) in all manure samples (Fig. 2). Information about additional elements including B, Ca, Fe, Mg, Mn, Na, and S in each manure sample is provided in Appendix A Table S3 and Figs. S1-S3. Several studies have reported concentrations of Zn and Cu in dairy manure similar to those reported here. A survey of twenty New York dairies found Zn and Cu concentrations in manure of 191 and 139 mg/kg, respectively (McBride and Spiers, 2001). Four dairy farms in China had mean Zn and Cu concentrations in manure of 144.4–225.5 mg/kg and 29.7–51.8 mg/kg, respectively (Li et al., 2019). Fresh cow manure from Spain had Zn and Cu concentrations of 262 and 23 mg/kg, respectively (Walker et al., 2003). In contrast to these studies, Brock et al. (2006) reported much greater concentrations of Zn (239 mg/kg) and Cu (409 mg/kg) in dairy manure than other studies, and notably, Cu levels were 1.7-fold greater than Zn.

The effects of manure application on metal concentration and bioavailability in soil vary according to metal species, manure and soil properties, and exposure time (Arnesen and Singh, 1998). Although Zn was more abundant in our manure samples, it is generally less mobile than Cu in soil and is often preferentially adsorbed over Cu on Al, Fe, and Mn hydrous oxides and soil exchange sites (Fageria et al., 2002). This increases the likelihood for Zn to accumulate in fields receiving annual manure applications (Imseng et al., 2019).

Bioavailability of Cu and Zn in soil depends on several factors including total metal concentration, cation exchange capacity, organic matter, and soil pH (Adrees et al., 2015). Although dairy manure's high organic matter content generally binds Cu and Zn (Leita et al., 1999) and restricts their leaching through soil, dissolved organic carbon or low molecular weight water-extractable carbon from manure can potentially facilitate transport of Cu and Zn through the soil profile as organometallic complexes (Brock et al., 2006; Guan et al., 2011; Lekfeldt et al., 2017; Merritt and Erich, 2003). However, such facilitated transport is most likely to occur in soils and organic amendments that have acid pH and since pH of dairy manure is typically alkaline, Cu and Zn leaching as organic complexes does not usually pose a significant concern.

Soil total Cu and Zn concentrations are strongly related to extent of manure application, but they are not necessarily correlated with the metal content or distribution in plants cultivated on the soil
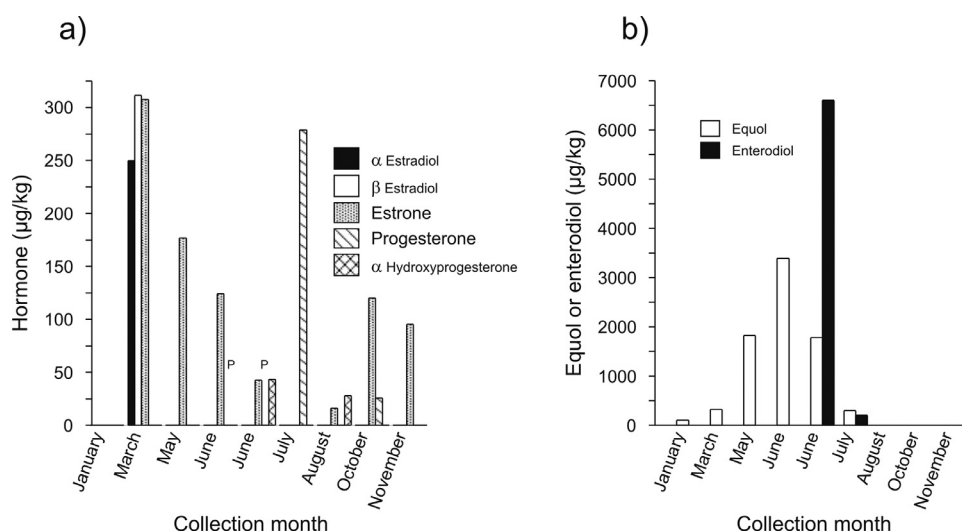
Fig. 3 – (a) Hormones detected in dairy manure composite samples in the respective sampling months. Progesterone (P) detections in June samples were below quantification limits; (b) Phytoestrogens detected in dairy manure in the respective sampling months.

(Mantovi et al., 2003). Across various crop species, Zn is generally more bioavailable than Cu in high pH soils. Plant responses to bioavailable soil Cu are highly variable. For example, research suggests that mechanisms exist in wheat (*Triticum aestivum*) to restrict Cu translocation from the roots and throughout the plant (Guan et al., 2011). In contrast, high total and extractable soil Cu had no effect on Cu concentration in bean (*Phaseolus vulgaris*) shoots (Senkondo et al., 2015). Also, increasingly high levels of bioavailable soil Cu resulting from applications of Cu-spiked dairy manure had no effect on the growth, yield, mineral concentration, or forage quality of corn (*Zea mays*) (Flis et al., 2010).

2.4. Organic contaminants

Five hormones were identified in the manure samples: 17β-estradiol, 17α-estradiol, estrone, progesterone, and 17α-hydroxyprogesterone. Estriol was not detected in any sample. Estrone was detected most frequently, in seven of the nine samples, and in the highest concentration, up to 307.4 µg/kg dried manure (Fig. 3). Progesterone was detected in four samples, but concentrations were below quantification limits in two of those samples (Fig. 3). There was a clear temporal distribution of hormone excretion (Fig. 3), with manure estrogen levels tending to be greater in spring samples and progesterone levels tending to be greater in summer and fall samples.

These hormone concentrations are consistent with the range reported by other studies on dry stacked dairy manure (Raman et al., 2004; Zheng et al., 2008). Although dairy cows produce only 17β-estradiol, their intestinal microbes produce 17α-estradiol. Consequently, both estradiol isomers are frequently reported in dairy manure (Gadd et al., 2010; Hanselman et al., 2003). Estrone is the degradation product of both estradiol isomers and is often the most frequently detected hormone in aged manure samples (Raman et al., 2004; Zheng et al., 2008), as is consistent with our results.

The distribution and concentration of hormones in dairy manure found in this study (Fig. 5) exemplify the variability among individual dairy operations. Reproductive performance of dairy cows, and thus steroid hormone production and excretion levels, is impacted by several factors including environmental conditions (De Rensis and Scaramuzzi, 2003; Hagevoort and Garcia, 2013), genetics, management practices, and animal health and nutrition (Walsh et al., 2011). Genetic selection for high-yielding milk breeds and increased use of growth-promoting pharmaceuticals (IDA, 2019) have caused milk yield per cow in the U.S. to steadily increase over the past 50 years. This can negatively impact cows' reproductive physiology, including decreased production and excretion of estrogen and progesterone (Rodriguez-Martinez et al., 2008; Walsh et al., 2011; Wiltbank et al., 2006). Man-

agement of dairy herd health and nutrition is especially challenging in large herds (>1500 cows), such as those found in Idaho's Magic Valley - the source of manure for this study (Rodriguez-Martinez et al., 2008).

The temporal distribution of hormones seen in our results may reflect herd management and calving cycles common at southern Idaho dairies, since the geographic region and its characteristic climate pattern affect dairy cow gestation variables (Norman et al., 2009). Although calf birthrate on commercial U.S. dairies peaks in September and is lowest in April, calving occurs year-round, which makes it challenging to decipher hormone excretion trends in manure (Norman et al., 2009). The method of manure collection used in this study inherently added significant variability since samples were taken from a central compost facility that receives manure from numerous regional dairies, each of which contributes uniquely to biological variability in the composite manure. Estrogen and progesterone excretion increases throughout gestation and peaks at birth (Desaulniers et al., 1989; Erb et al., 1968, 1977; Hanselman et al., 2003; Yost et al., 2014), though progesterone concentrations are typically much higher than total estrogens at all points in the reproductive cycle (Yost et al., 2014). The sharp peaks of estrogen levels in March and progesterone levels in July (Fig. 3) may correspond to increases in calf birthrates. However, concentrations of estrogen and progesterone are expected to follow similar temporal trends, as opposed to the dissimilar hormone concentrations seen in this study.

Although hormones are frequently found in dairy manure, they decompose rapidly in soil and decomposition rates increase in the presence of manure (Lucas and Jones, 2006). In a laboratory microcosm study investigating degradation of 17β-estradiol in a silt loam soil, Xuan et al. (2008) found that the degradation rate constant was proportional to the percentage of nonsterilized soil, illustrating the influence of soil microorganisms on 17β-estradiol degradation (Xuan et al., 2008). Additional studies indicate that estrone and 17β-estradiol strongly sorb to soil (Das et al., 2004), readily forming non-extractable and soil-bound residues across distinct agricultural soils (Casey et al., 2003; Colucci et al., 2001). The results of these studies suggest low potential for aqueous transport of estrogens through soil and low potential bioavailability.

Despite the limited mobility of estrogen in laboratory batch studies, surveys have consistently found them in surface water, ground water, and agricultural plots that receive manure applications (Bradford et al., 2008; Schuh et al., 2011; Yu et al., 2019). Estrogens have been shown to move to depths of 35 m under constant, concentrated influxes such as under dairy lagoons (Arnon et al., 2008; Bradford et al., 2008). Estrogen movement through soil is enhanced by colloid-facilitated transport following effluent application (D'Alessio et al., 2014). Moreover, low to-

tal organic carbon and presence of macropores in sandy soil significantly contribute to facilitated transport of hormones in the environment (D'Alessio et al., 2014).

The presence of hormones in agricultural soils can cause other environmental problems. Endocrine disrupting chemicals from land-applied manure may negatively impact organisms, such as lizards who displayed estrogenic contamination only on fields that received animal manure application (Verderame et al., 2016). Vegetative uptake of estrogens from manure-amended soils may potentially impact humans and other organisms via dietary intake (Adeel et al., 2018b; Zhao et al., 2019). Lettuce uptake of estrogens has also been shown to negatively impact plant physiology, including root growth and development, leaf and root biomass, and chlorophyll concentration (Adeel et al., 2018a).

Hormones in runoff from manure-amended fields can threaten aquatic organisms. However, the lowest estrogen concentration that results in an observable effect varies according to the species and age of the organism, the type of estrogen, and the sediment properties (Lasier et al., 2016; Leet et al., 2011; Sangster et al., 2014, 2016). Widespread application of dairy and poultry manure on agricultural fields nearby the Upper Conasauga River (USA) resulted in bioavailable estrogen concentrations in river sediments that were positively correlated with decreased growth of the amphipod *Hyalella Azteca* (Lasier et al., 2016). 17β-Estradiol located in Nebraska river sediments was not bioavailable to the fathead minnow (*Pimephales promelas*) (Sangster et al., 2014), but female fathead minnows exhibited reduced reproductive success following exposure to progesterone-spiked sand or silt loam river sediments (Sangster et al., 2016). However, sediment type differentially influenced fish exposure and biological response to steroid hormones because of the varying sorption capacities of sand and silt loam sediments (Sangster et al., 2016).

Phytoestrogens were detected in the highest concentrations of all studied ECs (Fig. 3). Equol was detected in six manure samples at a maximum concentration of 3396 µg/kg and enterodiol was detected twice at a maximum of 6604 µg/kg dried manure. There is a clear temporal distribution of phytoestrogen excretion in dairy manure, with both compounds peaking in June (Fig. 3), suggesting the inclusion of soy-based feeds in the herd diet during those months. The primary phytoestrogens in soy (*Glycine max*) are genistein and daidzein and the latter is known to be transformed by bacteria to equol in the intestinal tract of animals (Setchell and Clerici, 2010). Equol was detected in the highest concentrations among six phytoestrogens present in manure collected from several Swiss dairies (Hoerger et al., 2011). Similarly, a comprehensive analysis of a swine sow operation showed that equol concentrations were orders of magnitude greater than other phytoestrogens in urine and feces (Yost et al., 2014). Phytoestrogens have been detected in global surface waters and they, too, can impact aquatic organisms (Jarošová et al., 2015). A comparison of five phytoestrogens determined that equol caused the most endocrine effects on two different fish species (Latonnelle et al., 2002), and male Japanese medaka (*Oryzias latipes*) exposed to equol had a high occurrence of intersex, defined as simultaneous presence of male and female gonadal tissue (Kiparissis et al., 2003).

This is the first study to document the presence of the non-steroidal anti-inflammatory drug (NSAID) flunixin in solid stacked manure from regular dairy operations, although it has been detected previously in milk and dairy products (Chen et al., 2019; Kissell et al., 2012; Xie et al., 2015). Here, flunixin was found in three samples, twice near the detection limit and once at 136.9 µg/kg dried manure in the June 2016 sample (Fig. 4). Flunixin is used to treat inflammation and pain in hoofed livestock, including cows and horses in the United States. Flunixin has not been tested for endocrine-disrupting capacity, and it is not an antibiotic. Very little research has been done on the environmental transport or fate of flunixin. One study searching for race-promoting drugs in a racehorse stall found flunixin in the highest concentration of three NSAIDs on the barn walls, the dust on the rafters, and in the lagoon pond (Barker, 2008), suggesting that flunixin may be pervasive in the environment.

Flunixin, like most NSAIDs, is toxic to wildlife. In 2012, a wild Eurasian Griffon Vulture was found dead in Spain with elevated levels of flunixin from consuming livestock that had recently been given the drug (Zorrilla et al., 2015). This was the first recorded case of an NSAID overdose in vultures outside of Asia, and the first documented case of wildlife overdose from flunixin. In the 1990s, Asian *Gyps* vultures went nearly extinct from NSAID toxicity, with one species dropping by 99.9% in 15 years (Zorrilla et al., 2015). Flunixin residues are widely distributed throughout the musculature of dairy cows and despite maximum residue limits to ensure food safety, 71% of flunixin residue violations in the U.S. come from market dairy cows (Shelver et al., 2016; USDA, 2017). Frequent use and accumulation of flunixin in the environment and in dairy cows could prove dangerous to wildlife and human health.

2.5. Antibiotics

Antibiotics were found in six of the nine samples, and a single manure sample frequently had more than one type of antibiotic (Fig. 4). Two different sulfonamides (sulfadimethoxine and sulfamethazine) were detected in four manure samples (Fig. 4), and compounds in the tetracycline family (tetracycline, chlorotetracycline, and oxytetracycline) were detected four times (Fig. 4). Monensin was detected in seven of nine samples ranging from 20.4 to 801.27 µg/kg dried manure and once at a concentration below quantification limits (May 2016). Sulfamethoxazole, sulfathiazole, formononetin, biochanin A, and penicillin were not detected in any of the samples.

Concentrations of antibiotics in manure are expected to vary widely among dairies due to their different management practices. Additionally, manure sampled in this study was stored for unknown periods of time at individual dairies before it was brought to the composting facility. This partly explains the concentrations detected, such as with monensin, which varied from BDL to >800 µg/kg. The variety of compounds detected in these dairy manure samples is similar to what has been reported in other studies on solid dairy waste composition and the compounds are present in similar concentrations (Feng et al., 2016).

Dairies have stricter requirements than other food-animal production systems regarding pharmaceutical supplements, and the FDA permits very few pharmaceuticals to be administered during lactation. Monensin is one of the few veterinary drugs that the FDA has approved for use during lactation (Maron et al., 2013; Santos et al., 2019; Watanabe et al., 2008). It is used to promote lactation, prevent coccidiosis, and treat ketosis (Duffield et al., 2012; Fisher and Scott, 2008). Likely, this explains why monensin was the most frequently detected antibiotic in this study and detected in the highest concentrations. Globally, monensin is often detected in dairy manure solids, dairy wastewater (Watanabe et al., 2008), groundwater below dairies (Watanabe et al., 2008), and in surface water downstream from dairy operations (Fisher and Scott, 2008; Forrest et al., 2011; Kim and Carlson, 2006; Kurwadkar et al., 2013). For example, the presence of 27 livestock antimicrobials was surveyed in 23 agricultural streams in Alberta, Canada and monensin was the most frequently detected drug, found in 34% of the sites (Forrest et al., 2011). Other veterinary pharmaceuticals associated with dairies have been frequently found in the environment. A survey in Australia detected oxytetracycline, tetracycline, penicillin, and sulphasalazine in creeks and streams known to be downstream from dairy operations (Fisher and Scott, 2008).

Numerous factors influence the extent to which hormones and pharmaceuticals in the environment threaten human health. Dietary intake of foods grown in manure-amended soil is one route by which humans are exposed to contaminants of emerging concern. Plant bioavailability of contaminants of emerging concern is influenced by many factors, including soil texture, organic matter content, and compound type. For example, tomato and cucumber uptake of pharmaceutical compounds from wastewater irrigation was inversely proportional to soil organic matter content and increased with soil texture: clay loam < sandy loam < sandy (Goldstein et al., 2014). When grown in manure-amended and antimicrobial-spiked soil, maize (*Zea mays* L.) tissue accumulated greater sulfonamide concentration while tetracycline antibiotics mainly accumulated in the soil (Mullen et al., 2019).

2.6. Mobile genetic elements and ARGs

Monitoring the routes of spread of antibiotic resistance from diverse sources is becoming part of a strategy to fight antibiotic resistance (Berendonk et al., 2015). Class 1 integrons are genetic ele-
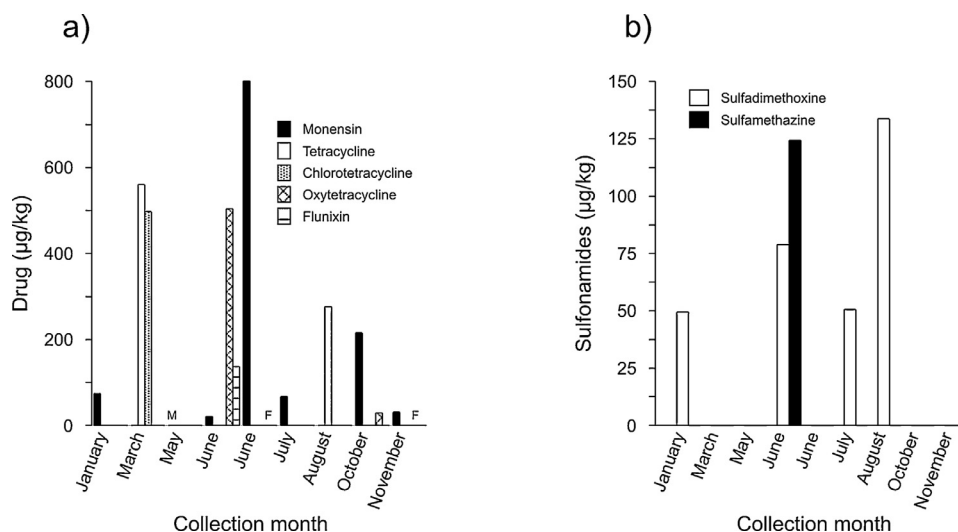
Fig. 4 – (a) Tetracyclines, monensin, and flunixin detected in dairy manure composite samples in the respective sampling months. Capital letters represent months in which concentrations of monensin (M) and flunixin (F) were detected, but at concentrations below quantification limits; (b) Sulfonamides detected in dairy manure in the respective sampling months.

Table 4 – Approximate copy number (in gene copies per gram) and normalized copy number per copy of 16S rRNA encoding gene (in parenthesis) of class 1 integron and sulfonamide resistance genes (*sul1* and *sul2*) in animal manures.

Origin	Class 1 integron	*sul1*	*sul2*	Reference
Dairy cow (Minnesota, USA)	5×10^8 (8×10^{-4})	NA	NA	Sandberg and LaPara, 2016
Dairy cow (Finland)	NA	NA (1×10^{-4})	NA	Ruuskanen et al., 2016
Dairy cow (Finland)	NA ($\tilde{}10^{-3}$)	NA (5×10^{-4})	NA	Muurinen et al., 2017
Dairy cow (Yangling, China)	8×10^7 (2×10^{-4})	5×10^8 (1×10^{-3})	2×10^9 (1×10^{-2})	Sun et al., 2016
Cattle fecal deposits (Canada)	NA	$\tilde{}10^8$	NA	Alexander et al., 2011
Dairy cow (Anhui province, China)	9×10^7 (7×10^{-4})	1×10^8 (9×10^{-4})	5×10^8 (4.2×10^{-4})	Peng et al., 2017
Dairy cow (Michigan, USA)	NA	1.5×10^8 (9×10^{-6} to 9×10^{-5})	NA	Munir and Xagoraraki, 2011
Dairy cow slurry (Estonia)	1×10^7 (1×10^{-4})	1×10^6 (1.5×10^{-4})	NA	Nõlvak et al., 2016

ments significantly involved in the rapid evolution and spread of antibiotic resistance (Cambray et al., 2010; Gillings, 2017b). They are considered biomarkers of anthropogenic impact (Gillings, 2017a, 2017b; Gillings et al., 2015; Stalder et al., 2012, 2013, 2014; Stedtfeld et al., 2017) and have been used in studies to assess anthropogenic impacts in soils and rivers (Aubertheau et al., 2017; Chen et al., 2016; Jechalke et al., 2014a, 2014b; Sandberg and LaPara, 2016), as well as in dairy cow manure from various regions of the world (Table 4). Sulfonamide resistance genes *sul1* and *sul2* constitute additional potential biomarkers for monitoring the spread of antibiotic resistance. Resistance to sulfonamides, some of the most used antibiotics in dairies, is highly prevalent in bacteria isolated from animals (Tadesse et al., 2012). Such resistance is often linked to class 1 integrons (Sánchez-Osuna et al., 2019).

Here we quantified class 1 integron integrase gene *intI1* and sulfonamide resistance genes *sul1* and *sul2* in all manure samples with copy numbers ranging from 1.2×10^7 to 1.8×10^9 copies/g of manure (Appendix A Fig. S4). The normalized copy numbers of class 1 integrons and sulfonamide resistance genes had the lowest variations of copy number among samples, especially for *sul1* where values were all ranged within one order of magnitude (Fig. 5). The resistance gene *sul1* was positively correlated with *intI1* (Spearman rank correlation coefficient *sul1* vs. *intI1* r = 0.52, P = 0.0064), indicating their genetic co-localization on the so called 'clinical integrons' as previously reported (Gillings, 2017a). However, here the correlation was rather weak compared to other studies (Jechalke et al., 2016; McKinney et al., 2018; Nõlvak et al., 2016; Sun et al., 2015). The *sul1* resistance gene was also

positively correlated with *sul2* (Spearman rank correlation coefficient *sul1* vs *sul2* r = 0.85, P = 4.2×10^{-8}), suggesting either a genetic linkage through a shared bacterial host or via a genetic element such as a plasmid, or selection (direct selection by sulfonamides antibiotics or indirect selection by metals). Measured copy numbers were similar to the ones found in the literature for the same source (Table 4). The consistency of the normalized gene copy number within all manure samples suggests that class 1 integrons and sulfonamide resistance genes are widespread across dairies within the studied area. We conclude that these genes constitute suitable biomarkers to assess the influence of dairy manure fertilization on the spread of antibiotic resistance in Southern Idaho.

The emergence of bacterial pathogens that are resistant to multiple antibiotics is largely caused by the sharing of resistance genes through horizontal gene transfer mediated by plasmids (Frost et al., 2005; Holmes et al., 2016). Plasmids are mobile genetic elements that replicate independently from the chromosome and can often transfer between bacteria by conjugation. Many carry the integrons described above (Zhang et al., 2018). For the first time, we quantified the occurrence of plasmids from several plasmid incompatibility groups in manure. These included plasmids recently found to emerge from farm settings such as those belonging to the incompatibility groups IncQ1 and IncI, which were reported to confer resistance to critically important drugs of last resort (Liu et al., 2016; Mollenkopf et al., 2017; Poirel and Nordmann, 2016). Despite the prominent role of plasmids in the emergence of multidrug resistance in pathogens, assessment of their pres-
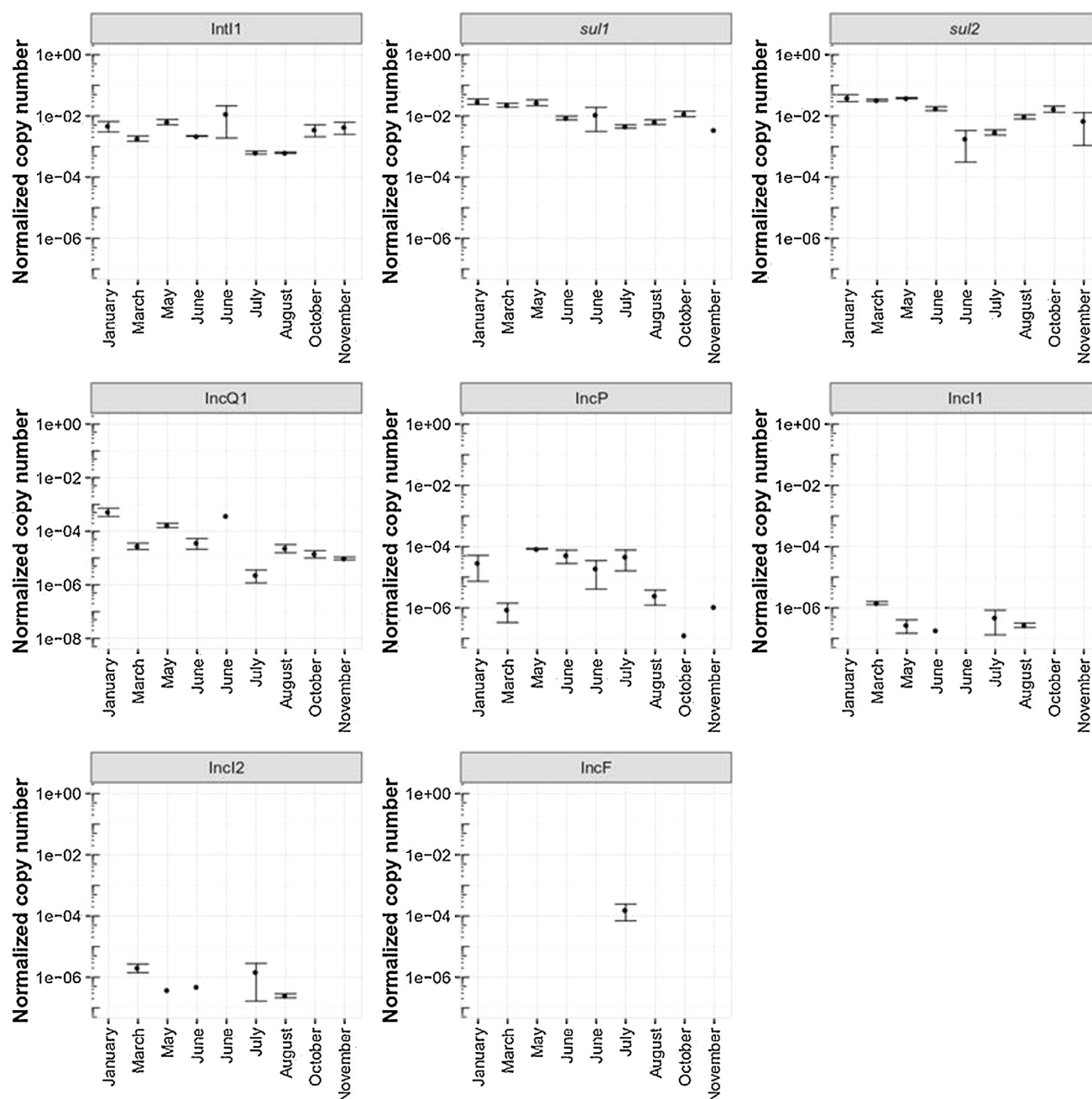
Fig. 5 – Normalized copy number per copy of 16S rRNA encoding gene of class 1 integron integrase genes (*intI1*), sulfonamide resistance genes (*sul1* and *sul2*), and IncP, IncQ1, IncI1, IncI2, and IncF* plasmids in dairy manure composite samples sampled at different times. Closed circles represent the means of three independent manure replicate samples and bars represent the standard deviations. *Not all IncF subgroups were targeted.

ence and spread via manure has received much less attention than ARGs or class 1 integrons. Here we developed a new set of primers and probe to quantify the IncQ1 plasmids and measured the copy numbers of the IncI1, IncI2, IncF, and IncQ1 plasmids in manure for the first time.

IncQ1 and IncP plasmids were detected in all samples, with copy numbers ranging from less than 1.8×10^5 to 1.1×10^8 copies/g of manure. The IncI1 and IncI2 plasmids were present in almost all samples at copy numbers below the limit of quantification. Finally, IncF plasmids were only detected and quantified in one sample of manure in July 2016. However, the primers and probes used here only target a fraction of the IncF plasmids (Blau et al., 2018); therefore, lack of de-

tection of IncF plasmids does not necessarily indicate their absence in the samples. The normalized copy number of IncQ1 plasmids was significantly correlated to *intI1, sul1*, and *sul2* resistance genes, suggesting a genetic linkage (Spearman rank correlation coefficient for IncQ1 vs. *intI1* was $r = 0.59$, $P = 0.0012$; for IncQ1 vs. *sul1* was $r = 0.70$, $P = 7.92 \times 10^{-5}$; for IncQ1 vs. *sul2* was $r = 0.48$, $P = 0.012$). IncQ-like plasmids have been reported in piggery manure (Smalla et al., 2000; Tietze, 1998; Wolters et al., 2016) and are frequently associated with the resistance gene *sul2* in animals and humans (Sundin and Bender, 1996). Our results indicate that the broad host range IncQ1 plasmids are widespread in dairy cow manure from Southern Idaho.

3. Conclusion

The manure samples evaluated here illustrate the wide variability in concentrations of nutrients and CECs in manure (Larney and Hao, 2007). While most of our results reflect general trends reported previously, including temporal fluctuations, a few new observations were made. Despite the wide range of N:P:K ratios, the median C:N ratio of 12:1 suggests that N will be readily plant available and thus dairy manure is a potentially good source of soil fertility. Manure concentrations of Cu and Zn reported herein were similar to those reported in other studies. The high organic matter content of dairy manure strongly binds the metals, thereby decreasing their bioavailability and mobility through soil. The manure hormone concentrations measured in this study were similar to those reported in the literature, and the temporal changes illustrate the large variability among dairy manures. Phytoestrogens were the most prevalent of all emerging contaminants detected in the manure samples and their high concentration in dairy manure is a potential concern because they can significantly disrupt endocrine function in aquatic organisms.

Veterinary pharmaceuticals and antibiotics were frequently found in the dairy manure samples. This is the first study to document the presence of flunixin, a non-steroidal anti-inflammatory drug, in solid stacked manure from commercial dairy operations. Monensin, one of the few antibiotics permitted in lactating cows, was found most frequently and in the greatest concentrations. While the distribution and concentration of antibiotics varied among manure samples, Class 1 integrons and IncQ1 plasmids that facilitate the transmission of ARGs were found in all samples. Given the widespread nature of these genetic elements in dairy manure, they could function as biomarkers for assessing the effect of land-application of dairy manure on antibiotic resistance spread in Southern Idaho.

Acknowledgements

Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.07.016.

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