ON-FARM FORAGE FERTILIZATION AND
CATTLE MANURE VALUE CHAIN CHARACTERIZATION IN VIETNAM,
LOW-INFRASTRUCTURE FIBER TECHNIQUE, AND
IMAGE ANALYSIS FOR ALFALFA-GRASS HARVEST MANAGEMENT

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Keenan Clay McRoberts, Ph. D.
Cornell University 2015

Crop-livestock systems play a key role in food and economic security for smallholder farmers worldwide. Research targeting farmer and agricultural extension educator-identified agroecological problems can help provide empirical solutions to these problems and improve access to evidence-based information. This dissertation includes components of four studies undertaken in Vietnam and New York State. The first objective was to assess the impact of forage fertilization with composted cattle manure and urea on dry matter yield and nutritive value of *Brachiaria* Cv. Mulato II forage in south-central coastal Vietnam. Highest forage yields were observed with combinations of urea N and compost. Partial nutrient budgets were negative, suggesting that the management system may not be sustainable over time. Few nutritive value effects were observed, suggesting that fertilization management for high yield is more feasible than management for high nutritive value. The second objective was to characterize cattle manure value chains originating in two Vietnamese communes in the south-central coast. Active seasonal manure trade took place between origin communes and end users (i.e., highland pepper and coffee farms, highland rubber plantations, and southeast coastal dragon fruit farms). Manure trade plays an important economic role for manure value chain participants and may affect sustainability of local nutrient management systems in manure origin and destinations. The third objective was to develop a low-infrastructure neutral detergent fiber technique for forage chemical analysis that could provide accessible technology for low budget laboratories such as those found in many developing countries. The technique was tested on a diverse set of temperate and tropical grasses and legumes to assess performance, which overall did not differ from a standard filter bag method (ANKOM). The fourth objective was to develop a field technique to reduce uncertainty in prediction of alfalfa and grass fractions in mixed stands in New York State. Local binary patterns in digital images were processed several ways to assess system potential to improve spring harvest timing and fiber estimates with equations requiring an accurate stand composition estimate. Current results suggest that the method can be useful in the field, but further testing is needed. Data and information generated in these diverse studies are useful to farmers, extension educators, and development practitioners, and also provide direction for future work.

BIOGRAPHICAL SKETCH

Keenan is the son of Wayne and Cathie McRoberts. He was raised on his family's farm and exotic animal ranch in western Nebraska. He received his B.S. in Biochemistry from the University of Nebraska-Lincoln in 2001. From 2001 to 2005, Keenan worked as an agricultural extension educator with the U.S. Peace Corps in a northern Nicaraguan community (Ococona), and as a Technical Specialist and Trainer in Estelí, the third largest city in Nicaragua. He completed his Master of Professional Studies in International Agriculture and Rural Development at Cornell University in 2010. He is Chairman of the Board of Directors for Help Educate, a nonprofit dedicated to improving access to higher education and leadership development for rural Nicaraguan youth. Keenan and Jacqueline Benson married in 2014 and now reside in Saint Louis, MO.

DEDICATION

Dedicated to my late mother, Cathie McRoberts,

whose positive attitude and zeal for life inspire me.

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Study specific acknowledgments including funding support are located at the end of each chapter.

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CHAPTER 1: UREA AND COMPOSTED CATTLE MANURE AFFECT FORAGE PRODUCTION AND SOIL FERTILITY IN SANDY SOILS OF VIETNAM

Abstract

Production of improved forages in backyard plots is important for sustainable growth of Vietnamese smallholder beef systems. Our objectives were to evaluate the effects of mineral nitrogen (urea) and composted cattle manure on *Brachiaria* Cv. Mulato II (*B. ruziziensis* x *B. decumbens* x *B. brizantha*) and soil fertility in Cát Trinh Commune, Bình Định Province. Study design was a randomized complete block on six farms (blocks), with treatments derived from the factorial combination of five rates of composted cattle manure (0, 4, 8, 12, and 24 Mg DM/ha per yr) and three urea rates (0, 60, and 120 kg N/ha per yr). The study was conducted from late 2010 to early 2013. Treatments were split-applied at 2-mo intervals, and grass was harvested at approximately monthly intervals. Harvest measurements included dry matter (DM) yield, forage nutritive value, live tiller count, canopy and maximum height, and plant counts. Soil was analyzed pre- and post-experiment. Highest yields were achieved with combined high compost and urea treatments, while urea and compost applied in isolation did not increase yield. Compost increased soil pH, organic matter, Ca, Mg, and Mn. The effect of compost and urea applications on post-experiment soil fertility depended on pre-experiment soil fertility for K, P, S, Mg, Zn, Mn, Cu, and organic matter, suggesting that the ability to maintain soil fertility is dependent on the interaction between soil organic and inorganic amendments and existing soil fertility. Highest farm yields were achieved on farms with higher pre-experiment soil fertility levels. Initial yield and tiller response to urea application was evident, but was not sustained over time where no compost was applied. We conclude that composted cattle manure combined with urea benefits grass yield and soil fertility, but relatively high application rates are needed for sustainable growth of Vietnamese smallholder beef systems on sandy soils.

1. Introduction

Animals raised on smallholder crop-livestock farms (1 to 5 head cattle per household) in south-central coastal Vietnam supply part of the growing demand for beef in urban areas (Parsons *et al.*, 2013). Cattle systems in the region are predominately semi-intensive (grazing and stall feeding) or extensive (grazing of communal land), with few household operations practicing only intensive stall feeding. Typical cattle management consists of supervised or unsupervised grazing on communal (and sometimes private) land and supplementary stall feeding with a basal diet of rice straw, peanut straw, and cultivated forage (e.g., *Pennisetum purpureum* Schumach.) or cut-and-carry native grasses and legumes from private and communal land (Parsons *et al.*, 2013).

In recent years, many farmers have initiated the transition toward semi-intensive operations to increase animal productivity (daily gain) by improving the quantity and nutritive value of forages and reducing maintenance energy expenditures incurred from walking to open grazing areas. A key component of this transition is cultivation of high yielding, high quality forages that are well-adapted to local climatic and soil conditions (Ba *et al.*, 2013; Parsons *et al.*, 2013; Stür *et al.*, 2013; Ba *et al.*, 2014). Production of improved forages in backyard plots also benefits farmers by reducing labor required for cattle production (Stür *et al.*, 2013; Khanh *et al.*, 2014). Supplementary forages used in appropriate combination with existing low-cost ingredients (e.g., rice straw) play an important role in animal productivity and income gains.

Information on the effect of management decisions on yield and quality of forages in sandy, tropical, rain-fed systems such as those located in the south-central Vietnam coast is scant (Devendra, 2002; Stür *et al.*, 2002). Important farm management decisions include establishment (via seed or vegetative material), harvest intervals, cutting height, irrigation, and fertilization.

Palm *et al.* (1997), Zingore *et al.* (2008), Goyal *et al.* (1999), and Kaur *et al.* (2005) suggest that a combination of locally available organic nutrient sources and fertilizer might be needed to enhance productivity in the tropics. Farmers in south-central coastal Vietnam typically use composted cattle manure combined with occasional inorganic fertilizer applications (urea and/or NPK blends) (McRoberts, Chapter 3). Farmers generally follow a "more is better" approach to composted manure applications based on plant color and vigor.

Sandy soils in Vietnam, similar to other tropical systems, pose a unique set of fertility constraints (Hoa *et al.*, 2010), including nutrient deficiencies and low soil organic matter (OM) concentrations (Neve *et al.*, 2009). Low organic matter contributes to low cation exchange capacity (CEC), low water holding capacity, and high potential nutrient losses due to runoff, leaching, volatilization, and denitrification. If perennial forage grasses with high nutrient uptake potential can be grown year-round, they can contribute to productivity of the herd while reducing nutrient loss to the environment. Farmers in the south-central region of Vietnam prefer grasses over legumes due to higher yield potential for small plot production (Stür *et al.*, 2013).

The objective of this study was to assess the effects of urea and composted cattle manure on forage yield and soil fertility via a multi-year (29-mo) on-farm field experiment. The effects of urea and composted cattle manure in the study on forage quality are reported in McRoberts (Chapter 2).

2. Methods

2.1 Cát Trinh commune characteristics

The experiment was conducted between 2010 and 2013 in Cát Trinh Commune, Phù Cát District, Bình Định Province in south-central coastal Vietnam. Cát Trinh borders the district capital of Ngô Mây (14°0′2″ N, 109°2′38″ E) on the north and east. Sandy soils in the commune belong to the Arenosols Group. Annual rainfall in the region is about 1,200 mm, with over 70% falling during the rainy season (September to December) (Table 1). Average monthly temperatures range from 23 to 31°C (Table 1). Precipitation during the experiment period was generally consistent with recent patterns in the study region (Table 1). Rainfall was above average in November 2010, causing flooding and plant loss during the plant establishment period on one of the farms. Rainfall was below average in September 2011 and October and November 2012, contributing to overall below average annual precipitation. Dry season rainfall was low and consistent with historical patterns. Most rainfall occurred during the September to December rainy season in 2011 (76%) and 2012 (55%). Temperatures were within normal ranges.

Table 1 Nine-year historical precipitation means and monthly precipitation and temperatures were extracted for experiment years from http://www.wunderground.com for the Quy Nhơn weather station, located approximately 35 km southeast of Cát Trinh Commune. Precipitation during active experiment months is highlighted in gray.

Month	Mean (8/2004 to 7/2013)	SD (8/2004 to 7/2013)	2010	2011	2012	2013	Max Temp		Min Temp	
							Mean	SD	Mean	SD
	Precipitation (mm)						ºC			
Jan	45	45	44	11	68	75	26	0.6	22	0.4
Feb	13	12	0	8	18	39	27	0.7	22	1.1
Mar	36	41	0	37	10	12	28	1.7	23	1.4
Apr	24	25	7	5	67	27	30	0.9	25	1.0
May	78	85	39	56	9	230	33	0.8	27	0.4
Jun	25	9	38	15	37	29	34	0.5	28	0.4
Jul	47	44	50	79	94	122	34	0.5	27	0.3
Aug	63	65	99	16	60	75	34	0.6	28	0.3
Sept	245	312	54	96	207	64	32	0.6	26	0.3
Oct	278	157	285	291	104	165	30	0.4	25	0.2
Nov	294	221	742	290	86	224	28	1.8	24	1.0
Dec	79	95	12	60	49	6	27	1.7	23	0.9
Total	**1,226**	**583**	**1,370**	**964**	**809**	**1,069**				

Location selection was based on the presence of the Australian Centre for International Agricultural Research-funded Project SMCN/2007/109, entitled, "Sustainable and Profitable

Crop and Livestock Systems for South-central Coastal Vietnam". Project presence simplified access to commune farms, including provincial and commune permission requirements. It furthermore increased the odds of adequate experiment tracking and eventual dissemination of results due to close and frequent contact with commune extension educators and rural development professionals operating in the region. This experiment complemented existing research and extension initiatives, and responded directly to problems identified by smallholder farmers and priorities revealed during baseline project surveys (Hoa, 2009; Parsons *et al.*, 2013). The field experiment was established on smallholder farms in collaboration with farmers and local extension educators to ensure farmer participation in the experiment and potentially higher acceptance of research results, which has been a noted problem in Southeast Asia (Devendra, 2002; Stür *et al.*, 2002). Farmers contributed to implementation and experiment management decisions, and managed weed control and plot irrigation as needed. Farmer practice was adopted for most aspects of experiment management.

2.2 Farm selection and experiment preparation

Commune extension staff recommended six cattle owners based on the following criteria: (1) non-participants in previous Australian Centre for International Agricultural Research-funded initiatives, (2) interest in participation, (3) availability of a well-drained, level parcel near the household (13 m x 13 m or equivalent), and (4) access to irrigation. Recent farm field histories included: (1) *Pennisetum purpureum* Schumach. cultivation, (2) vacant with household waste and ash accumulation, (3) cassava and eggplant production, (4) cassava production, (5) peanut production, and, (6) vacant lot. Consequently, the six fields represented sandy soils varying in soil fertility levels (Table 2). Pre-experiment (2010) soil samples at a depth of 15 to 25-cm indicated low organic matter, low nutrient concentrations, and acid soils (Table 2).

In July 2010, experimental areas were prepared for planting according to farmer practice, using animal traction and handheld hoes. Weeds were controlled with handheld hoes as needed (one to two times per month) throughout the experiment. A 1.2-m high woven wire fence was placed around each experimental area to prevent animal entry. Pesticides were not applied during the experiment.

Table 2 Pre-experiment soil fertility levels at 15 to 25-cm depth on six farms in Cát Trinh Commune. Elements are Mehlich-3 extractable nutrients.

Parameter	Overall Mean (n=96)	Overall SD	Farm Means (n=16)					
			Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
CEC[a] ($cmol_+$/100 g)	1.3	0.60	2.4	1.4	1.3	0.9	0.9	1.0
pH (1:1 soil:water)	5.7	0.66	6.0	5.5	6.2	5.8	4.6	5.9
Field pH[b]	5.3	0.29	5.5	5.2	5.6	5.3	5.2	5.2
OM (g/kg)[c]	3.5	2.1	5.2	4.5	2.1	1.6	5.1	2.5
S (mg/kg)	9.1	4.47	10.1	9.9	6.3	5.6	17.0	5.6
P (mg/kg)	37	27.3	67	41	68	22	14	12
Ca (mg/kg)	176	68.3	269	204	190	126	116	151
Mg (mg/kg)	29	23.3	71	19	28	17	21	16
K (mg/kg)	36	36.2	91	41	32	11	31	12
Na (mg/kg)	23	13.1	43	20	19	19	21	17
B (mg/kg)	0.18	0.19	0.25	0.38	0.24	0.01	0.15	0.04
Fe (mg/kg)	116	75.2	180	142	153	28	169	27
Mn (mg/kg)	7	7.5	14	7	16	1	2	5
Cu (mg/kg)	1.6	1.29	3.3	2.3	1.6	0.6	1.1	0.6
Zn (mg/kg)	2.7	5.43	4.4	7.1	1.5	0.4	1.0	1.6
Al (mg/kg)	280	179.7	375	346	235	116	540	72
NO_3-N (mg/kg)	3	2.6	5	5	1	1	5	2
NH_4-N (mg/kg)	8	4.1	5	15	7	6	8	7

[a] CEC = cation exchange capacity.
[b] The Cornell pH test kit (Cornell Nutrient Analysis Laboratory, Ithaca, NY) was used to assess field soil pH.
[c] Soil organic matter (OM) was determined by loss-on-ignition at 360°C (Schulte and Hopkins, 1996).

Preliminary reviews from Cát Trinh farmers participating in forage varietal selection experiments suggested *Brachiaria* Cv. Mulato II (*B. ruziziensis* x *B. decumbens* x *B. brizantha*) was the most appropriate grass given its popularity among farmers (Ba *et al.*, 2013; Ba *et al.*, 2014), drought tolerance, adaptation to acid soils, and high nutritive value relative to other C_4 forages (Argel *et al.*, 2007; Inyang *et al.*, 2010; Vendramini *et al.*, 2012). A high density Mulato II seedbed (> 500 plants/m^2, covered) was established at farm 3 in August 2010. Young plants (15 to 30-cm maximum height) were transplanted into experimental areas approximately 4 weeks after seedbed establishment. Starter fertilizer (20-20-15; N-P_2O_5-K_2O) was applied at 175 kg/ha just before transplanting (early September 2010), and again in October 2010 after transplanting. Twenty Mulato II plants were established in each 2 x 2-m plot (50-cm row spacing and 40-cm plant spacing). Inter-plot buffers (1 m) and outer borders of experimental areas contained a single row of Mulato II (40-cm spacing between plants). Replanting occurred where needed during the first two months after establishment (September and October 2010) to obtain the desired density in the 2 x 2-m plots (50,000 plants/ha).

2.3 Experimental design

The randomized complete block design on six smallholder farms (blocks) included fifteen treatments derived from factorial combination of five composted manure rates (0, 4, 8, 12, and 24 Mg DM/ha per yr) and three urea rates (0, 60, 120 kg N/ha per yr). Treatment 16 consisted of the highest compost rate and 80 kg K_2O/ha per yr in 2011. The highest urea rate was added to this treatment from January 2012 onward. Buffers received 4 Mg DM/ha per yr compost.

Farm 3 provided all the composted manure to ensure fertility treatment consistency across farms. Preparation consisted of daily removal of cattle manure and rice straw orts from stalls and placement in an uncovered pile for approximately 45 d. The pile was turned at least twice prior to use. Compost application was done to achieve rates of 0, 40, 80, 120, and 240 kg N/ha per yr based on an initial assessment of average compost nutrient content. Actual N rates (Table 3) differed due to variation in composted manure DM and N concentrations over time (Table 4).

Compost treatments were selected to meet Mulato II N requirement (240 kg N/ha per yr) with the highest compost application rate (and without urea application), according to J. Corfield (personal communication, July, 2010).

Yearly treatments were split into six applications applied by hand at approximately 2-mo intervals directly after harvest. Treatments were surface-applied in year one and incorporated (top 0 to 15 cm) with handheld hoes from year two onward to reduce potential for nutrient transfer across plots and prevent nutrient loss, which should not impact yield based on an application method experiment in the same region (Van *et al.*, 2014). Two months post-transplanting (November 2010), all plants were cut uniformly at 25-cm above ground level using a handheld sickle to promote the development of vertical tillers. Initial fertilization treatments were applied at that time.

Table 3 Actual nitrogen (N) treatment rates were combinations of urea N and N in composted cattle manure. Error is standard deviation and exists due to variation in compost composition over time during the experiment.

Compost Rate (Mg DM/ha per yr)	Urea Rate (kg N/ha per yr)		
	0	60	120
0	0	60	120
4 ± 0.7	46 ± 12.4	106 ± 12.4	166 ± 12.4
8 ± 1.3	92 ± 24.9	152 ± 24.9	212 ± 24.9
12 ± 2.0	138 ± 37.3	198 ± 37.3	258 ± 37.3
24 ± 4.0	276 ± 74.7	336 ± 74.7	396 ± 74.7

2.4 Sampling protocols

2.4.1 Forage samples

A 30-d cutting interval and 15-cm cutting height were targeted to achieve high yield and nutritive value without negatively impacting persistence (Inyang *et al.*, 2010; Vendramini *et al.*, 2014). Plots were harvested at approximately monthly intervals (36 d with SD ± 8.6 d) between

January 2011 and April 2013 (24 harvest events in total). Harvest was done initially at 15-cm cutting height above ground level using handheld sickles. Cutting height increased during the experiment due to thickening crowns. Labor availability, weather, and drying oven space prevented the harvest of all plots on the same day, but farms were harvested within two to four days and in the same sequence at each harvest window. The cutting intervals in 2011 (39 d with SD ± 12.3 d) were longer and more variable than intervals for the rest of the experiment (33 d with SD ± 3.3 d), due to a missed October 2011 harvest because of flooding in the study area (maximum harvest interval = 63 d).

Table 4 Means and standard deviations for composition of composted cattle manure from the supplying farm in Cát Trinh Commune on a dry matter basis were calculated from 14 treatment applications, and each treatment application was an average of 3 subsamples.

Parameter	**Mean**	**SD**
Dry matter (%)	50	8.4
Organic matter (g/kg)[a]	261	99
Mineral matter (g/kg)[a]	739	99
C:N[b]	12.8	2.3
Total N (g/kg)	12	3
Organic N (g/kg)	11	3
P (mg/kg)	2733	1302
K (mg/kg)	6750	4105
Ca (mg/kg)	9988	3581
Mg (mg/kg)	3088	1094
Na (mg/kg)	955	661
S (mg/kg)	2090	516
B (mg/kg)	14	4
Fe (mg/kg)	4266	2196
Mn (mg/kg)	791	129
Cu (mg/kg)	27	25
Zn (mg/kg)	110	25

[a] Organic matter was estimated by loss-on-ignition at 550°C for 2 h (Peters *et al.*, 2003) and mineral matter was calculated as *100 – %OM*.
[b] 1.724 factor was applied to convert organic matter (OM) to C, based on the assumption that Loss by Ignition = OM, and OM contains 58% organic carbon [Factor of Wolff, as cited in Pribyl (2010)].

At harvest, live tiller count from three plants per plot were counted by subdividing crowns into four equal parts and counting live tillers in one quarter. The same plants were measured throughout the experiment. Grass maximum height (tallest extended grass blade), canopy height, and plants per plot were recorded. The recording of plant vigor (10-point scale from 1=least vigorous to 10=most vigorous), water logging (6-point scale from 0=no standing water to 5=severe plant death due to extreme inundation for prolonged periods), and leaf attrition (6-point scale from 0=no death to 5=80 to 100% of leaves and tillers dead) began in the 2011 rainy season (October) and was completed for 15 data collection periods. Other factors potentially impacting plot growth during a given harvest were recorded (e.g., chicken damage, cattle damage, cattle urine runoff into plots, accidental cutting). Flooding destroyed some plots on farm 2 in fall 2010 and again in 2011. Replanting was required and there were missing yield data during and after these periods for the affected farm.

For each plot, all fresh forage biomass was collected and weighed. Subsamples (200 to 300 g) were collected every other harvest event for forage quality assessment while in between smaller subsamples (20 to 50 g) were collected to determine DM concentration. Subsamples were dried at 60°C in a forced-air oven until a stable weight was obtained to determine DM concentration. Forage quality in the experiment is reported in McRoberts (Chapter 2). Luxury N consumption was assumed for N nutrition index values above one (Lemaire *et al*., 2008), which are also reported in McRoberts (Chapter 2).

2.4.2 Composted cattle manure samples

Three representative subsamples (150 g each) of the composted cattle manure were collected during each treatment application period. The subsamples were dried to stable weight at 60°C in a forced-air oven, and ground to pass a 2-mm screen in a Retsch cutting Mill (Germany) at Huế University of Agriculture and Forestry. Brookside Laboratories, Inc. (New Bremen, OH) analyzed samples as described in Peters *et al.* (2003). Total N was determined using an Elementar Vario Max (Elementar Analysensysteme, Hanau, Germany), and minerals were determined using a CEM Mars Express microwave (CEM Corporation, Matthews, NC) with digest analyzed in a Thermo Scientific iCAP 6500 inductively coupled plasma-atomic emission spectrometer (Thermo Electron Corp., Waltman, MA). Ammonium-N and NO_3-N were determined using a Lachet QuickChem 8000 flow injection calorimetric analyzer (Lachat Instruments, Loveland, CO). Organic matter (OM) was estimated by loss-on-ignition at 550°C for 2 h (Peters *et al.*, 2003) and mineral matter was calculated as *100 – %OM*.

2.4.3 Soil samples

Pre-experiment soil samples (n=96) were collected in August 2010 using a handheld trowel at a depth of 15 to 25-cm below ground level (Table 2), and post-experiment samples in May 2013 at two depths (0 to 15-cm and 15 to 25-cm). In each plot, three samples were gathered on a diagonal line across the plot and combined into a single composite sample for each depth. Samples were air-dried, litter was removed, and soil was passed through a 2-mm sieve. Brookside Laboratories, Inc. (New Bremen, OH) determined soil pH (1:1 H_2O) (McLean, 1982), using an AS-3000 Dual pH Analyser, soil OM by loss-on-ignition at 360°C (Schulte and Hopkins, 1996), Mehlich-3 (Mehlich, 1984) extractable P, K, Ca, Mg, S, Na, B, Mn, Cu, Zn, and Al in a Thermo Scientific iCAP 6500 inductively coupled plasma-atomic emission spectrometer (Thermo Electron Corp., Waltman, MA), and inorganic N (NH_4-N and NO_3-N via 1 N KCl cadmium reduction) using a Flow Injection Analyser (FIAlab Instruments, Inc., Bellevueo, WA) (Dahnke and Johnson, 1990). Values below the limit of detection were replaced by *limit of detection/2* to enable statistical analysis. The Cornell pH test kit (Cornell Nutrient Analysis Laboratory, Ithaca, NY) was used to assess field soil pH pre-experiment, mid-experiment, and after the final harvest.

2.5 Partial nutrient balance and nitrogen recovery calculations

Partial nutrient balances for N, P, and K were calculated as *inputs - outputs*, where *inputs* included nutrients applied in fertilizer and compost treatments and *outputs* were nutrients accumulated in harvested forage. Nutrient outputs were calculated by multiplying back-transformed mean yearly treatment yields (geometric means) by mean nutrient concentration for each treatment in September 2011 samples and December 2012 samples. Partial balance estimates by treatment were then calculated as the average of the September 2011 and December 2012 balances. Nitrogen recovery was calculated according to the difference method of Jokela and Randall (1997) as *% N recovery = [(N uptake in treatment$_i$ – N uptake in zero-N control) / total N applied in treatment$_i$] x 100.*

2.6 Composted manure incubation test

A composted manure incubation study tested potential for short-term immobilization of soil nitrates. A New York State fine-loamy soil (pH = 7.6, OM = 64 g/kg, Morgan NO_3-N = 17 mg/kg) (100 g) was amended with 1 g or 10 g representative compost (4 replicates) in plastic cups with perforated lids. Cups were arranged in a completely randomized design and incubated at 23°C for two weeks in a dark chamber. Soil moisture was maintained between 70% and 75% of field capacity during the incubation. Post-incubation, samples were dried to stable weight at 50°C in a forced-air oven. Subsamples were analyzed for KCl extractable nitrate (Griffin *et al.*, 1995) in an Easychem Analyzer (Systea Scientific, Oak Brook, IL).

2.7 Statistical methods

Mixed model procedures analyzed results from the multi-year experiment using PROC MIXED (SAS Institute Inc., 2011). Models included fixed effects of compost rate, urea rate, and their interaction, and block (farm) as a random effect. Models with repeated measures (DM yield, live tiller count, grass maximum height, grass canopy height, grass vigor, leaf attrition) were selected with block and block x treatment random effects to account for dependence of observations within farms and within plots over time. Repeated measures models were selected with fixed effect covariates including rainfall (cumulative rainfall in each growth period), mean temperature (mean daily temperature during growth period, computed as midpoint between maximum and minimum temperature), and harvest interval (days from previous harvest to current harvest). Additional candidate fixed effect covariates included the number of plants in each plot (plant count) and principal components derived from pre-experiment soil fertility factors generated in JMP PRO 11.2.0 (SAS Institute Inc., 2013) from individually significant soil fertility factors in a selected yield model. The first principal component was used as a candidate covariate in mixed models, and was calculated as: *Soil PC1 = -9.617 + 0.879 x Cation Exchange Capacity + 1.076 x Field pH + 0.00706 x Ca + 0.0339 x Na + 0.0588 x Mn + 0.0944 x* NO_3*-N* . Fixed effect covariates and interactions (up to 2-factor interactions) were individually removed from the model at $P > 0.05$ in a stepwise manner, and the model was run again until only main treatment effects, significant covariate effects and interactions remained. Kenward-Roger's approximation was used to calculate denominator degrees of freedom for models with repeated measures. Mean differences among treatments were declared at $P \leq 0.05$ using Tukey's statistic to control the family-wise error rate for multiple comparisons. Main effect interactions were also evaluated using the slice option in the lsmeans statement of PROC MIXED, which uses an F test to assess interaction slices, but does not control for family-wise error.

Yield decline was tested by comparing paired means between the first and second experiment years with a t-test and via assessment of the yield difference between years one and two. Two breakdowns were evaluated: annual DM yield from November 2010 to November 2011 versus annual DM yield from November 2011 to November 2012, and April 2011 to April 2012 versus April 2012 to April 2013. Mean differences were calculated as *year 2 DM yield – year 1 DM yield*. Seasonal yield variation was tested with a treatment x season interaction in a model with season in lieu of harvest period and weather variables. Seasonal variation in live tiller count was evaluated using a model with year and harvest period nested in year in lieu of harvest interval, climatic factors, and the soil principal component.

The effect of compost and urea treatments on soil fertility factors was evaluated in a mixed model with fixed effects of compost, urea, compost x urea, and pre-experiment soil fertility, and block as a random effect. Interactions between pre-experiment soil fertility factors and main effects were also evaluated to determine if soil fertility responses depended on initial soil fertility levels. One-tailed t-tests were used to assess paired mean differences between pre-experiment and post-experiment soil fertility data, with Bonferroni correction used to identify significant mean differences.

Non-normal distributions for DM yield, live tiller count, canopy height, and maximum height were identified by visual assessment of quantile-quantile plots and distribution of model residuals. Log transformation was applied for DM yield while a square root transformation was used for live tiller count, canopy height, and maximum height. Geometric means and 95% confidence intervals were calculated for transformed data in lieu of true means and standard errors.

3. Results

3.1 Mulato II yield and development

3.1.1 Dry matter yield

Dry matter yield responded to urea and to the urea x compost interaction, but not to compost alone (Table 5). When composted manure was not applied, urea application did not increase DM yield (P=0.6528), and when urea had not been applied, yield did not respond to compost application (P=0.9167) (Table 6). Urea did increase yield when compost was applied at 4, 12, and 24 Mg DM/ha per yr and compost application increased yield when urea was applied at 60 kg N/ha per yr, with a similar trend at 120 kg N/ha per yr (Table 6). Differences among main effect interactions were detected (Figure 1). The highest combined rate of compost and urea resulted in higher yield than the no compost or urea control, the plots where compost was applied at 12 or 24 Mg DM/ha per yr without urea application, where the highest rate of compost was applied but only 60 kg N/ha per yr as urea, and where 60 kg N/ha per yr was applied as urea without compost addition (Figure 1). In general, lowest numerical yields were obtained for compost only treatments or urea only treatments (Figure 1).

Table 5 Type 3 tests of fixed effects for a mixed model estimating log-transformed dry matter yield in the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Compost	4	69.5	1.1	0.3629
Urea	2	69.6	9.74	0.0002
Compost x Urea	8	69.5	2.32	0.0287
Rainfall	1	1987	129.69	<0.0001
Mean Temperature	1	1987	0.02	0.8926
Plant Count	1	2055	60.33	<0.0001
Harvest Interval	1	1987	177.28	<0.0001
Soil Principal Component	1	1811	64.67	<0.0001
Rainfall x Mean Temperature	1	1987	104.96	<0.0001
Rainfall x Harvest Interval	1	1987	149.19	<0.0001
Mean Temperature x Soil Principal Component	1	1988	102.79	<0.0001

Table 6 Tests of effect slices in log-transformed dry matter yield model for urea x composted manure interaction. This test evaluates the effect of urea at each level of compost and the effect of compost at each level of urea.

Compost (Mg DM/ha per yr)	Urea (kg N/ha per yr)	Num DF	Den DF	F Value	Pr > F
0		2	69.2	0.43	0.6528
4		2	69.5	3.26	0.0444
8		2	69.4	1.57	0.2157
12		2	69.7	3.44	0.0378
24		2	69.7	10.03	0.0001
	0	4	69.6	0.24	0.9167
	60	4	69.5	3.23	0.0171
	120	4	69.3	2.31	0.0662

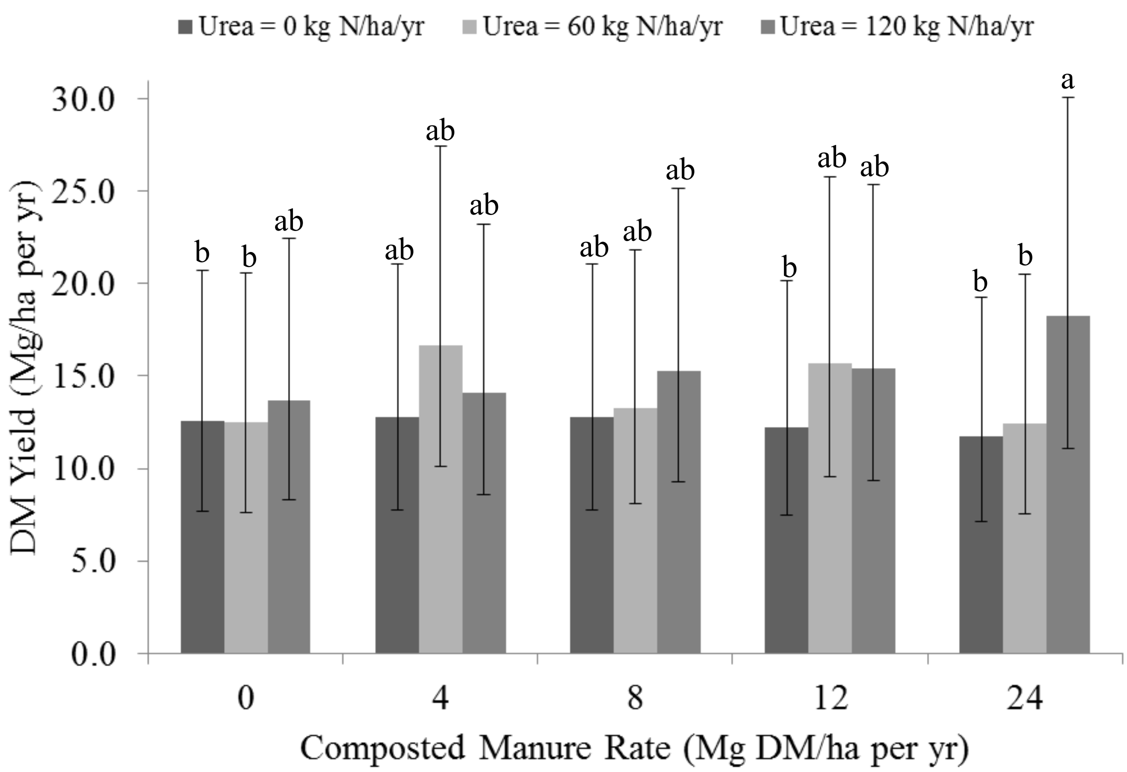

Figure 1 Back-transformed dry matter (DM) yield least squares means (geometric means) with 95% confidence intervals for composted manure x urea treatment combinations. Treatments not connected by the same letter are significantly different.

Results were similar for a model including harvest period in lieu of weather factors and harvest interval. However, differences were not detected for the compost x urea interaction (P=0.2058). Treatment differences or lack thereof did not vary among harvest periods.

Climatic effects (rainfall and temperature), plant count, harvest interval, pre-experiment soil fertility, and significant interactions impacted yield, and were captured in their respective covariates in statistical models (Table 5). These variables did not interact with compost or urea treatments, indicating that while these factors affected yield, treatment response did not depend on other measured factors. Yield declined from the first to second year of the experiment (P<0.0001). Yield changes did not depend on compost or urea treatments. Highest forage yields occurred during the late dry season from approximately April to August when some rainfall occurred and temperatures were at peak levels. Yield declined throughout the rainy season and initial stages of the dry season.

Overall yield differences were higher between April 2012 to April 2013 and April 2011 to April 2012 (-4.1 Mg DM/ha per yr) than between November 2011 to November 2012 and November 2010 to 2011 (-3.6 Mg DM/ha per yr). All farms except one experienced a yield decline in the April comparison, while all farms but two experienced a yield decline in the November comparison. For two farms, the yield decline was large (April to April: Farm 3 = -8.3 Mg DM/ha per yr and Farm 6 = -10.6 Mg DM/ha per yr; November to November: Farm 6 = -9.9 Mg DM/ha per yr and Farm 3 = -11.1 Mg DM/ha per yr). At farm 2, where flooding occurred in fall 2010 and fall 2011, yield in year 2 was higher than the first year yield, perhaps due to the later re-establishment period for affected plots (less nutrient depletion from plant growth) and potential soil nutrient transfer during flooding. Seasonal differences in yield magnitude were observed, but treatment effects did not depend on season (rainy or dry) (P=0.9848).

The 16th treatment containing potassium yielded numerically lower than the highest compost x urea treatment combination, but the mean yield was not different from the mean yield of any other treatment. This was consistent for all response variables, and therefore, results for this treatment will not be reported or discussed further.

Large yield differences were observed among farms (Table 7), ranging from an average total yield of 6.7 to 26 Mg DM/ha per yr. In all models discussed above and below, farm-to-farm variation explained a large proportion of residual variance (51% in yield model, 34% in live tiller count, 37% in canopy height, 45% in maximum height, and 18% in vigor model). Large differences in farm yields can be attributed to variable farm plot histories and pre-experiment soil fertility levels. High farm-to-farm variability generated results that are potentially more representative of variation in sandy Vietnamese soils and sandy tropical soils, and more generalizable. Despite farm-to-farm variation, treatment effects were consistent as determined by the absence of interactions with compost and urea main effects. Furthermore, block x treatment (plot) explained only 6% of residual variance in the yield model, 8% in the tiller count model, 2% in the canopy height model, 4% in the maximum height model, and 5% in the vigor model.

Table 7 Back-transformed dry matter yield least square means (geometric means) and 95% confidence intervals for block (farm). Means not connected by the same letter are significantly different.

Farm	Geometric Mean	Lower 95% CI (Mg DM/ha per yr)	Upper 95% CI
3	26.0 a	23.6	28.6
1	15.7 b	13.7	18.0
2	15.0 b	13.6	16.5
5	13.5 b	12.2	14.9
6	9.0 c	8.2	10.0
4	6.7 d	6.1	7.5

3.1.2 Live tiller count

Live tiller counts responded similarly to treatments as did DM yield (Table 8). Pairwise correlation between live tiller count and DM yield was strong and positive (r=0.65). Live tiller count increased with urea application, but not compost, and there was a trend (P=0.0616) for a compost x urea interaction. Live tiller count for the highest urea rate (120 kg N/ha per yr) was higher than where no urea had been applied (Figure 2). Tests of effect slices (Table 9) indicated that, similar to DM yield, live tiller count did not respond to compost application when urea was not added, nor to urea when compost was not added. Live tiller count increased with urea application at the highest compost rate, with a similar trend observed when compost was applied at 4 Mg DM/ha per yr and 12 Mg DM/ha per yr. Live tiller count responded to compost only at the intermediate urea rate (60 kg N/ha per yr), but response was inconsistent and driven by high tiller counts when compost was applied at 4 Mg DM/ha per yr.

Table 8 Type 3 test of fixed effects for a mixed model estimating square root-transformed live tiller count in the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Compost	4	69.4	1.32	0.2729
Urea	2	69.5	6.13	0.0035
Compost x Urea	8	69.4	1.98	0.0616
Rainfall	1	1992	108.66	<0.0001
Mean Temperature	1	1992	14.39	0.0002
Plant Count	1	2061	17.37	<0.0001
Harvest Interval	1	1992	24.87	<0.0001
Soil Principal Component	1	1819	35.35	<0.0001
Rainfall x Mean Temperature	1	1992	112.81	<0.0001
Mean Temperature x Harvest Interval	1	1992	24.03	<0.0001
Mean Temperature x Soil Principal Component	1	1992	59.19	<0.0001

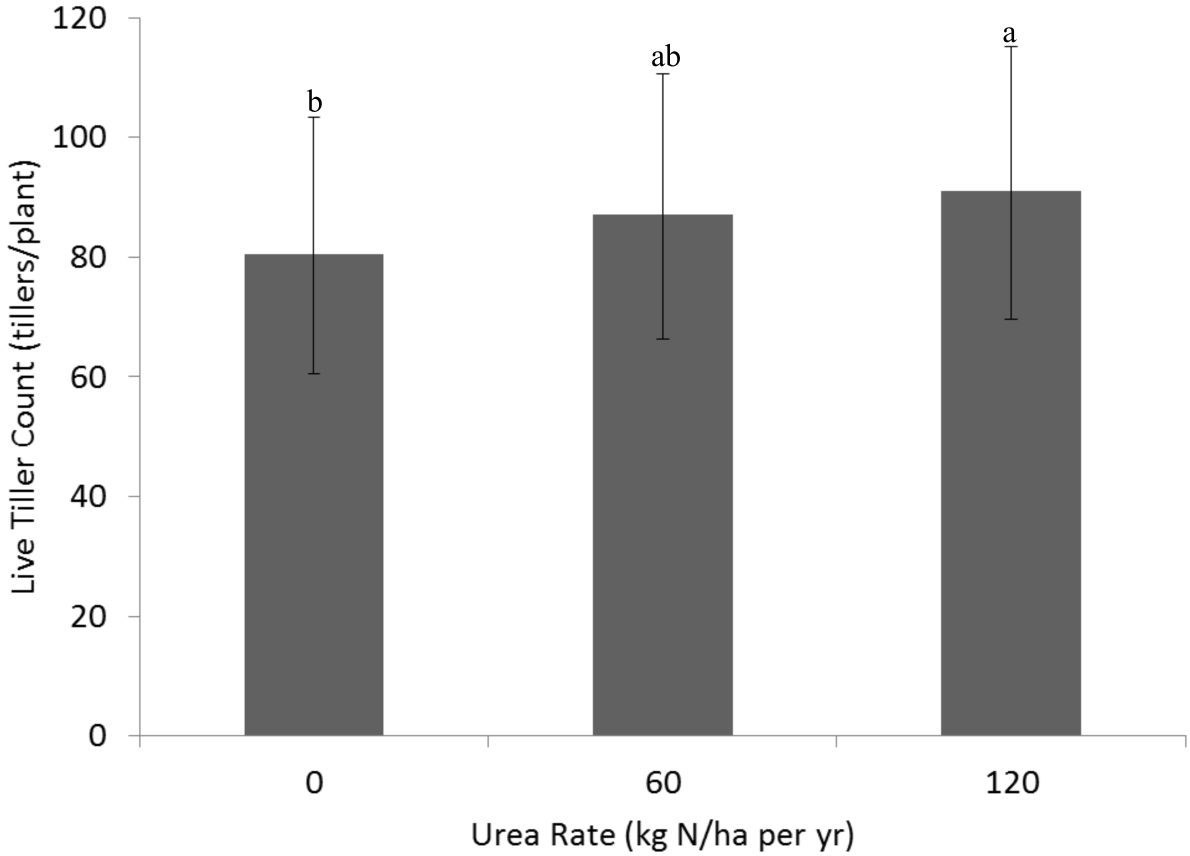

Figure 2 Back-transformed live tiller count least squares means (geometric means) and 95% confidence intervals for urea treatments. Treatments not connected by the same letter are significantly different.

Table 9 Tests of effect slices in tiller count model for urea x composted manure interaction. This test evaluates the effect of urea at each level of compost and the effect of compost at each level of urea.

Compost (Mg DM/ha per yr)	Urea (kg N/ha per yr)	Num DF	Den DF	F Value	Pr > F
0		2	69.1	0.38	0.6867
4		2	69.4	2.94	0.0593
8		2	69.3	0.96	0.3893
12		2	69.7	2.97	0.0580
24		2	69.7	6.57	0.0024
	0	4	69.6	0.26	0.9045
	60	4	69.4	3.01	0.0238
	120	4	69.3	2.01	0.1025

Live tiller count increased throughout the year until September, before declining from October to early in the subsequent year (Figure 3). Rainy season negatively impacted tiller count, which recovered during the dry season in the two full years observed. The range in tiller count declined from the first year of the experiment to the second year, but overall live tiller count did not decline from the first year to second year of the experiment.

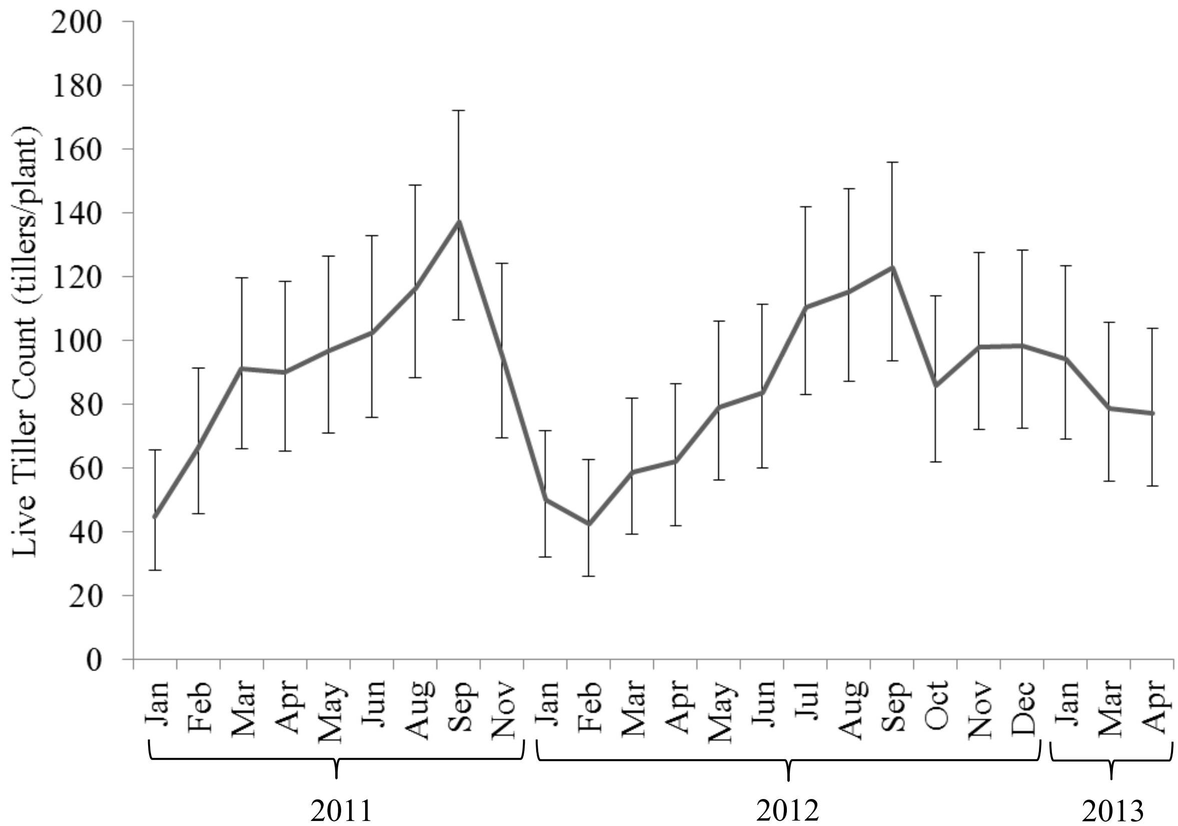

Figure 3 Back-transformed least squares means (geometric means) with 95% confidence intervals for live tiller count by harvest period during the experiment.

3.1.3 Canopy and maximum height, and plant vigor

Canopy height and maximum height responded to compost and urea (Table 10, Table 11). A urea x compost interaction effect was not detected. Canopy height and maximum height models were similar, although covariate interactions differed. Canopy height was higher for the two highest rates of compost than without compost, and maximum height was higher in the highest compost rate than without compost and at the intermediate rate (8 Mg DM/ha per yr) (Table 12). Canopy height was higher for the highest rate of urea than without urea, while urea application rate did not impact maximum height.

Table 10 Type 3 test of fixed effects for a mixed model estimating square root-transformed canopy height in the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Compost	4	67.4	5.74	0.0005
Urea	2	67.5	3.02	0.0554
Compost x Urea	8	67.3	0.98	0.4568
Rainfall	1	1989	293.75	<0.0001
Mean Temperature	1	2031	0.03	0.8697
Plant Count	1	2031	6.88	0.0088
Harvest Interval	1	1990	51.22	<0.0001
Soil Principal Component	1	2049	102.09	<0.0001
Rainfall x Harvest Interval	1	1989	122.71	<0.0001
Mean Temperature x Plant Count	1	2035	5.74	0.0167
Mean Temperature x Harvest Interval	1	1990	69.76	<0.0001
Mean Temperature x Soil Principal Component	1	1991	118.3	<0.0001

Table 11 Type 3 tests of fixed effects for a mixed model estimating square-root transformed maximum leaf height in the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Compost	4	68.7	5.41	0.0008
Urea	2	68.7	3.19	0.0472
Compost x Urea	8	68.6	1.21	0.3063
Rainfall	1	1990	89.82	<0.0001
Mean Temperature	1	2022	7.69	0.0056
Plant Count	1	2019	15.98	<0.0001
Harvest Interval	1	1990	125.01	<0.0001
Soil Principal Component	1	2016	107.19	<0.0001
Rainfall x Mean Temperature	1	1990	109.46	<0.0001
Harvest Interval x Soil Principal Component	1	1991	12.95	0.0003
Mean Temperature x Soil Principal Component	1	1991	122.14	<0.0001
Mean Temperature x Plant Count	1	2022	15.51	<0.0001

Table 12 Back transformed least squares means (geometric means) for grass canopy height and maximum leaf height, sorted by canopy height means.

Compost Rate (Mg DM/ha per yr)	Canopy Height[ab] (cm)	Maximum Height[ab] (cm)
24	46.4 a	66.4 a
12	44.7 a	63.8 ab
8	43.8 ab	62.8 b
4	43.8 ab	63.5 ab
0	42.1 b	60.8 b
Urea Rate (kg N/ha per yr)		
120	45.1 a	64.9 a
60	43.9 ab	62.7 a
0	43.5 b	62.8 a

[a] Means not connected by the same letter are significantly different.
[b] Mean comparisons for compost and urea are independent.

Compost and urea addition explained differences in plant vigor (Table 13). The highest combined rate of compost and urea resulted in greater plant vigor than for the no-compost or urea control, plots where compost was applied at 4 Mg DM/ha per yr without urea, where urea was applied at 60 kg N/ha per yr without compost, and where urea was applied at 60 kg N/ha per yr and compost at 8 Mg DM/ha per yr (Table 14). Leaf attrition did not respond to compost and urea treatments.

Table 13 Type 3 test of fixed effects for a mixed model estimating plant vigor in the experiment.

Effect	Num DF	Den DF	F Value	Pr > F
Compost	4	67.9	2.66	0.0398
Urea	2	68	5.67	0.0053
Compost x Urea	8	67.9	1.77	0.0986
Rainfall	1	1321	2.01	0.1561
Mean Temperature	1	1324	5.22	0.0225
Plant Count	1	1321	20.69	<0.0001
Harvest Interval	1	1324	12.42	0.0004
Soil Principal Component	1	1393	19.34	<0.0001
Rainfall x Harvest Interval	1	1321	22.47	<0.0001
Mean Temperature x Harvest Interval	1	1324	5.89	0.0154
Mean Temperature x Soil Principal Component	1	1321	29.06	<0.0001

Table 14 Least squares means for vigor. Vigor was assessed on a 10-point continuous scale from 1=least vigorous to 10=most vigorous.

Compost Rate (Mg DM/ha per yr)	Mean[ab]	SE
24	6.4 a	0.34
12	6.3 ab	0.34
8	6.1 ab	0.34
4	6.2 ab	0.34
0	5.8 b	0.34
Urea Rate (kg N/ha per yr)		
120	6.4 a	0.33
60	6.1 ab	0.33
0	6.0 b	0.33

[a] Means not connected by the same letter are significantly different.
[b] Mean comparisons for composted manure and urea are independent.

Yield was positively correlated with live tiller count (r=0.65), maximum height (r=0.63), canopy height (r=0.56), and vigor (r=0.53). Canopy height, maximum height, and vigor were the only plant indicators that responded directly to compost treatments (Table 10, Table 11, Table 13). Grass in plots receiving high compost rates grew taller and was more vigorous than grass grown with low rates of compost and urea. The tallest, most vigorous plants occurred in the highest compost x urea treatment combination.

3.2 Partial nutrient balances and nitrogen recovery

Overall harvested plant tissue nutrient concentration means were 24 g N/kg DM, 3.8 g P/kg DM, 21.2% g K/kg DM, 3.5 g Mg/kg DM, and 6.9 g Ca/kg DM, and the overall N nutrition index was 0.78. Average partial N, P, and K balances were negative on five of the six farms. Farms with better soil fertility indicators yielded higher, but also had more negative partial N balances. Partial N balance (Table 15) was most negative for treatments containing low N inputs, and progressively became less negative with additional N applications as urea and compost. Only the highest compost rate achieved partial N balances that were close to zero or positive (Table 15). Partial P and K balances by farm followed a similar pattern to N. All P and K balances were negative (Table 15). Treatments receiving higher rates of compost had numerically higher overall P balances. Potassium balances were very negative, impacted more by yield than by compost rates, reflecting the fact that compost K inputs (0, 3.5, 6.9, 10.4, and 29.8 kg K/ha per yr) were low relative to K removed in harvested forage (~290 kg K/ha per yr).

Overall partial N balances on lower yielding farms were positive (farm 4) or less negative (farms 5 and 6) than higher yielding farms. Overall partial P and K balances were negative on all farms, but were less negative for lower yielding farms. In general, pre-experiment soil fertility was better for the three farms (farms 1, 2, and 3) with the most negative balances and highest yields (Table 2, Table 7). However, residual soil nutrients are unlikely to entirely account for the observed negative balances in Arenosols with low CEC (1.3 $cmol_+$/100 g with SD ± 0.57) and low organic matter (0.35% with SD ± 0.21) (Table 2).

Table 15 Partial N, P, and K balances by composted manure and urea treatment combinations sorted by treatment. Balances were calculated using overall dry matter yield means for treatments (from section 3.1.1) and treatment N concentrations from September 2011 and December 2012 samples.

Compost Rate Mg DM/ha per yr	Urea Rate kg N/ha per yr	N Balance kg N/ha per yr	P Balance kg P/ha per yr	K Balance kg K/ha per yr
24	120	-67	-44	-380
24	60	32	-21	-262
24	0	-8	-19	-243
12	120	-113	-44	-328
12	60	-168	-44	-317
12	0	-153	-32	-261
8	120	-155	-49	-309
8	60	-160	-41	-277
8	0	-208	-39	-262
4	120	-181	-48	-272
4	60	-307	-59	-339
4	0	-264	-45	-263
0	120	-202	-49	-280
0	60	-236	-47	-276
0	0	-312	-47	-240

Greatest N recovery occurred in plots receiving the highest rate of urea (ranging from 5% to 42%) (Table 16). Highest overall N recovery was observed with 60 kg N/ha per yr urea x 4 Mg DM/ha per yr compost (79% and 112%, depending on nutritive value period). Nitrogen recovery in 60 kg N/ha per yr urea x 4 Mg DM/ha per yr compost treatment was also higher than the three remaining treatments receiving urea at 60 Mg DM/ha per yr (21% and 33% recovery, depending on nutritive value period). Other treatment N recoveries were similar to those obtained in the no compost and no urea control plots.

Table 16 Nitrogen recovery based on overall treatment yields and N rates in September 2011 and December 2012.

Compost Rate (Mg DM/ha per yr)	Urea Rate (kg N/ha per yr)	Nitrogen Recovery[a] Sept 2011 (%)	Nitrogen Recovery[a] Dec 2012 (%)
4	0	1.1	-10.8
8	0	-13.1	-13.0
12	0	-10.7	-20.4
24	0	-8.5	-12.4
0	60	-13.5	-42.2
4	60	78.5	112.2
8	60	0.5	-0.6
12	60	21.4	32.7
24	60	3.0	-7.8
0	120	10.6	5.0
4	120	23.4	17.9
8	120	21.0	30.3
12	120	22.4	23.0
24	120	34.0	42.2

[a] Nitrogen recovery was calculated according to Jokela and Randall (1997) as *% N recovery = (N uptake in treatment$_i$ – N uptake in zero-N control) / total N applied in treatment$_i$.*

3.3 Soil fertility

3.3.1 Incubation study results

The compost incubation study confirmed that addition of composted cattle manure at a high rate resulted in rapid N mineralization and nitrification as expected for material with C:N of 13:1 (Table 17). The incubation study did not provide evidence of N immobilization and the C:N ratio of the compost did not suggest that either. However, at the lower application rate, no increase in soil nitrate was determined. The latter supports the hypothesis that the lack of a yield response to compost addition in the farm experiments could be due to an insufficient increase in available N with the compost applications used in this study.

Table 17 Nitrate-N extracted from compost-amended soil, control, and non-incubated soil in a laboratory incubation study.

Treatment (g dry compost added to 100 g dry soil)	N Treatment equivalent (kg N/ha)	Soil Nitrate-N[a] (mg/kg)	SE
10	2,735	161.5 a	3.36
1	273.5	15.7 b	3.36
0	0	19.5 b	3.36
non-incubated soil	-	15.2 b	3.36

[a] Treatments connected by the same letter are not significantly different.

3.3.2 Soil fertility

Pre-experiment farm soil fertility levels were consistent with yield differences observed in the experiment (i.e., pre-experiment soil nutrient concentrations were higher for high yielding farms). All treatment means (n=6 for each compost x urea interaction rate) were numerically higher in post-experiment samples in the 15 to 25-cm stratum than pre-experiment samples at the same depth for OM and NH_4-N, and most means were numerically higher post-experiment for Na and Fe (Appendix 1 Table A1). More than half of post-experiment treatment means were also numerically higher than pre-experiment for Cu and Mn. All treatment means were numerically lower in post-experiment samples at the 15 to 25 stratum than pre-experiment samples at the same depth for S, P, K, and NO_3-N, and most were lower post-experiment for pH, Mg, Al, Ca, and CEC. Significant mean difference (Bonferonni correction) was detected only in soil Na for compost applied at 12 Mg DM/ha per yr without urea, which was higher post-experiment (Appendix 1 Table A1).

Models estimating soil fertility parameters in the 15 to 25-cm stratum (*post-experiment level – pre-experiment level*) indicated that compost impacted differences for soil Al (P=0.0189, ordered 12, 24, 4, 8, and 0, with 12 > 8 and 0) (Appendix 1 Table A2). Urea application decreased soil OM (P=0.0036, ordered 0, 60, 120, with 0 and 60 > 120) and tended to affect Mg (P=0.0898, ordered 0, 60, 120, no significant differences) (Table 18). A compost x urea interaction was detected for K (P=0.0413, no significant differences), with a tendency to impact pH (P=0.0595), Zn (P=0.0816), and NH_4^+ (P=0.0985).

Pre-experiment soil factors (PSF) most highly associated with yield were Mn (r=0.48), P (r=0.44), Fe (r=0.33), exchange capacity (r=0.32), and Ca (r=0.32). Many pre-experiment soil fertility factors were significant in multivariate models with compost and urea main effects (Appendix 1 Table A3). Models selected from main effects, PSFs and significant interactions indicated the importance of pre-experiment soil fertility on treatment responses for some soil fertility parameters (Table 18). The effect of compost and urea (main effects) on post-experiment soil S, K, Mg, Zn, Mn, and OM depended on their respective PSFs for both soil depths. Compost and urea effects on soil Cu depended on Cu PSF in the 0 to 15-cm stratum, while only Cu PSF emerged from the 15 to 25-cm stratum. The effect of compost and urea on soil CEC and P depended on PSF in the 15 to 25-cm stratum, while only PSF mattered in the 0 to 15-cm stratum. The effect of compost and urea on soil pH at 0 to 15-cm and NH_4-N at 15 to 25-cm did not depend on PSF, although it was in the model. Only PSF emerged from models estimating Fe, Al, and NO_3-N at both depths, CEC, P, NH_4-N, Na, and field pH at 0 to 15-cm, and Ca, pH, and Cu at 15 to 25-cm. Pre-experiment soil fertility was not significant for Ca at 0 to 15-cm or for Na and field pH at 15 to 25-cm. Farm explained greater than 40% of residual variation in soil fertility models at both depths for all factors except P, Zn, and CEC.

Table 18 Soil chemical models in 0 to 15-cm and 15 to 25-cm strata selected from candidate fixed effects of compost (C), urea (U), compost x urea (C x U), pre-experiment soil fertility for each parameter sampled at 15 to 25-cm depth (PSF), and significant interactions, with block included as a random effect.

Parameter[b]	0 to 15-cm depth		15 to 25-cm depth	
	Model Effect	Farm (%)	Model Effect	Farm (%)[c]
S (mg/kg)	C x U x PSF[a]	82	C x U x PSF[a]	85
Mg (mg/kg)	C x U x PSF[a]	87	C x U x PSF[a]	89
Zn (mg/kg)	C x U x PSF[a]	28	C x U x PSF[a]	16
Mn (mg/kg)	C x PSF[a]	87	C x U x PSF[a]	49
OM (g/kg)	C x U x PSF[a]	65	U x PSF[a]	77
K (mg/kg)	C x PSF[a]	51	C x PSF[a]	60
Cu (mg/kg)	C x U x PSF[a]	62	PSF	53
CEC ($cmol_+$/100 g)	PSF	31	C x U x PSF[a]	46
P (mg/kg)	PSF	52	C x U x PSF[a]	28
NH_4-N (mg/kg)	PSF	66	PSF C x U[a]	42
pH (1:1 soil:water)	C PSF	88	PSF	75
Fe (mg/kg)	PSF	88	PSF	70
Al (mg/kg)	PSF	62	PSF	58
NO_3-N (mg/kg)	PSF	43	PSF	46
Ca (mg/kg)	C x U[a]	75	PSF	49
Na (mg/kg)	PSF	47	C x U[a]	49
Field pH	PSF	59	C	62
B (mg/kg)	NS	-	NS	-

[a] Where interactions were significant, only highest level interactive effect or effects are shown, although lower level terms were also in the model.
[b] Each row represents a single model for each soil depth.
[c] Percentage of residual variance explained by farm in each model is indicated.

Compost addition increased soil pH in both soil strata (Figure 4, Appendix 1 Table A3), although pH for the 12 Mg DM/ha per yr compost rate was lower than the 24 Mg DM/ha per yr rate. Post-experiment soil pH was highest where compost had been applied, although pH decreased numerically in most plots relative to pre-experiment levels (Appendix 1 Table A1). Pre-experiment soil pH (1:1 soil:water) was higher than post-experiment pH for all treatments in the 15 to 25-cm stratum, which suggests minor soil acidification during the experiment. The difference between post- and pre-experiment pH was less for the highest compost treatment than 12 Mg DM/ha per yr. Post-experiment pH was impacted by compost treatments. Soil pH in the 0 to 15-cm stratum for the highest compost rate (pH=5.58) was higher than without compost (pH=5.37) and 12 Mg DM/ha per yr (pH=5.33) (Figure 4). Response to compost in the 15 to 25-cm stratum indicated that pH for the highest compost rate (pH=5.53) was higher than for the 12 Mg DM/ha per yr (pH=5.24) treatment. Overall, the deeper layer was slightly more acidic than the topsoil layer (Figure 4).

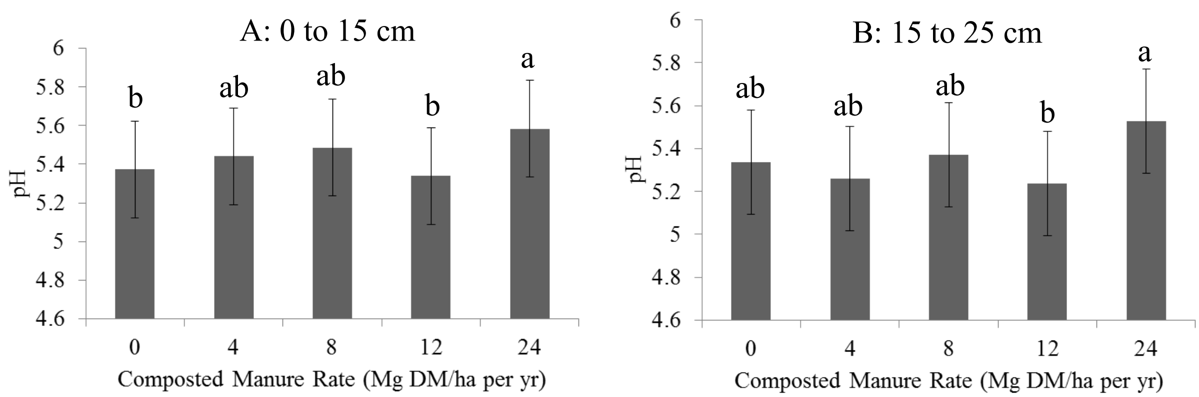

Figure 4 Post-experiment pH least squares means for composted manure treatment in 0 to 15-cm (A, P = 0.0071) and 15 to 25-cm strata (B, P = 0.0460) were extracted from models containing pre-experiment soil pH (Appendix 1 Table A3). Error bars are 1 SE of the mean. Bars connected by the same letter are not significantly different.

Compost increased soil OM in the 0 to 15-cm stratum (Figure 5, Appendix 1 Table A3). Urea application decreased OM in the 15 to 25-cm stratum (Figure 5, Appendix 1 Table A3). Organic matter for the highest urea rate was lower than the intermediate rate and plots not receiving urea, possibly explained by high mineralization rates in this stratum combined with limited OM addition to the soil in the form of plant litter. Organic matter in the 15 to 25-cm stratum for treatment plots increased by 0.8 g/kg to 3.8 g/kg from pre-experiment to post-experiment levels (Appendix 1 Table A1).

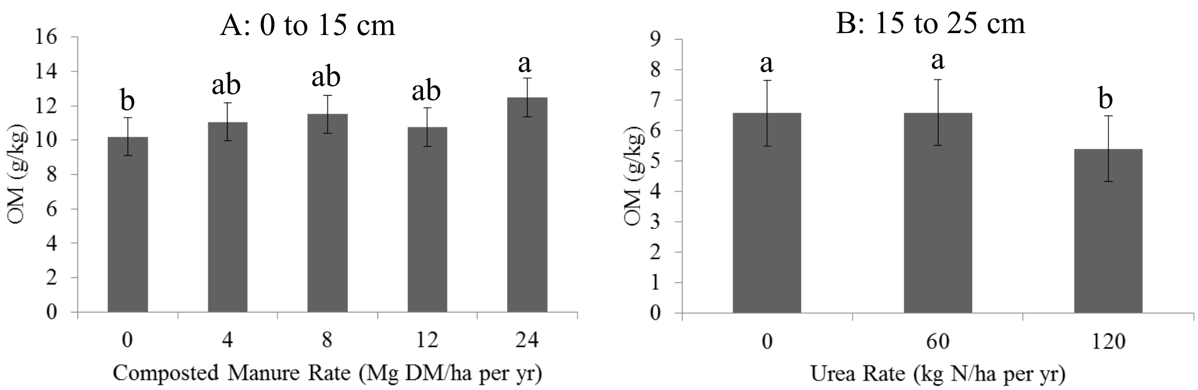

Figure 5 Post-experiment soil organic matter (OM) least squares means for composted manure rates in 0 to15-cm stratum (A, P = 0.0087) and least squares means for urea rates in the 15 to 25-cm stratum (B, P = 0.0035) were extracted from models containing pre-experiment soil OM (Appendix 1 Table A3). Error bars are 1 SE of the mean. Bars connected by the same letter are not significantly different.

Consistent with the OM effect, post-experiment Ca in the 0 to 15-cm stratum increased steadily with added compost (Figure 6), and likely contributed to buffering capacity (Figure 6, Appendix 1 Table A3). Calcium was higher for the highest compost rate than all other compost treatments. Compost x urea interaction was also observed (results not shown), but the interaction response was inconsistent. Calcium also increased numerically with applied compost in the 15 to 25-cm stratum.

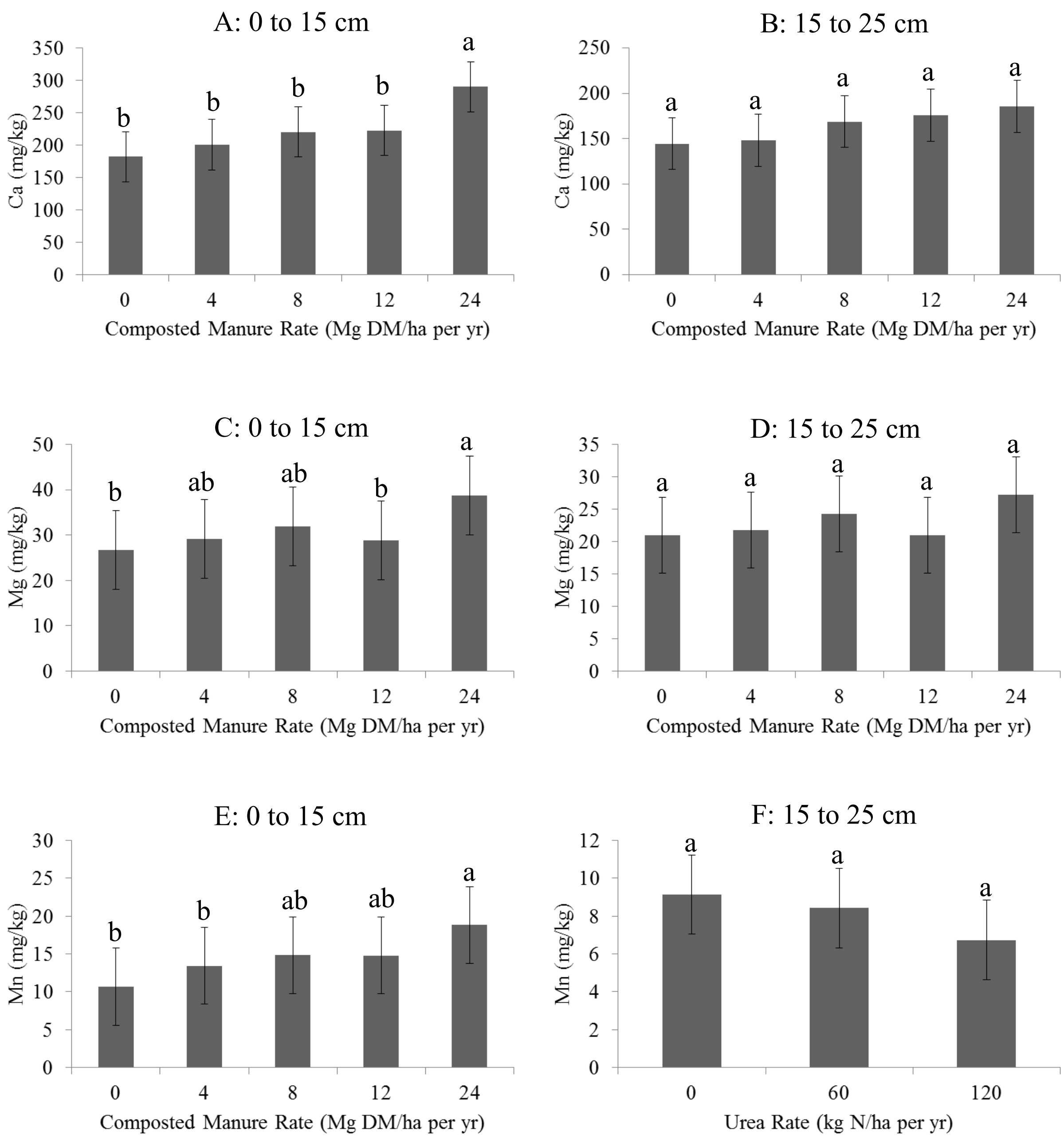

Figure 6 Post experiment soil Ca least squares means for composted manure rates in the 0 to 15-cm (A, P<0.0001) and 15 to 25-cm strata (B, P = 0.1873), Mg least squares means in the 0 to 15-cm (C, P = 0.0015) and 15 to 25-cm (D, P = 0.0542) strata, and Mn least squares means for compost treatments in the 0 to 15-cm stratum (E, P = 0.0002) and for urea treatments in the 15 to 25-cm stratum (F, P=0.2015). Error bars are 1 SE of the mean. Bars connected by the same letter are not significantly different. Models contain pre-experiment soil fertility factors (Appendix 1 Table A3).

Soil Mg increased with compost treatments in the 0 to 15-cm stratum, and a trend was observed in the 15 to 25-cm stratum (Figure 6, Appendix 1 Table A3). Similar to soil pH, Mg for the highest compost rate was numerically higher than all other treatments and soil Mg tended to increase with compost application (excepting the 12 Mg DM/ha per yr plots).

Soil Mn in the 0 to 15-cm stratum increased with compost addition, and Mn for the highest compost rate was higher than 4 Mg DM/ha per yr and plots not receiving compost (Figure 6, Appendix 1 Table A3). Similar to OM, Mn at 15 to 25-cm depth decreased with added urea, although the effect was not significant (P=0.2015).

Soil Na responses in the 15 to 25-cm stratum were probably due to an outlying treatment response. The compost x urea interaction effects for soil Na and soil NH_4-N in this stratum were inconsistent (results not shown). Pre-experiment soil Al levels tended to be higher than post-experiment levels in the 15 to 25-cm stratum (Appendix 1 Table A1). Soil Al for compost applied at 12 Mg DM/ha per yr was higher (lower mean difference) than 8 Mg DM/ha per yr and without compost (Appendix 1 Table A2). This suggests soil Al depletion during the experiment, especially for plots receiving no or a low rate of compost. Compost effect on Al was not detected in statistical analyses of post-experiment data. However, in general soil Al levels were numerically higher when less compost was applied, especially in the 15 to 25-cm stratum.

Observed soil fertility effects could be a function of pre- or post-harvest sampling time. Pre-experiment samples were collected in August 2010 and post-experiment samples in early May 2013. These soil sampling time frames were both during the dry season but rainfall was slightly higher leading up to August 2010 sampling than May 2013. Soil moisture near sampling times (not measured) could have affected mineralization rates and outcomes.

4. Discussion

4.1 Yield and agronomic components

Application of urea was most effective when combined with compost application, possibly reflecting greater overall N availability and release of P, K, secondary macro-, and micronutrients during compost mineralization. The combination of highest yields when both compost and urea were applied at the highest rates and a lack of a response to urea where no compost was applied suggests that (1) not only N but also other nutrients are yield limiting in these sandy soils, and (2) compost application at the rates in this study supplied insufficient amounts of N.

The overall yield decline over time is consistent with a decrease in available N as well, reflecting negative partial N balances. Such lack of N is also supported by N nutrition index levels that were consistently below 1 (McRoberts, Chapter 2). Peak Mulato II yields in this experiment were higher or equivalent to those reported elsewhere in the literature (González *et al.*, 2011; Pizarro *et al.*, 2013), but lower than observed for Mulato II in a forage varietal selection experiment in the same region (25.7 Mg DM/ha per yr) (Ba *et al.*, 2013), and lower than the yield average of 29.1 Mg DM/ha per yr for three south-central coastal provinces (Ba *et al.*, 2014). Higher inorganic N fertilization and shorter experiment duration may be responsible for higher yields in the Ba *et al.* (2013; 2014) experiments. Nitrogen concentrations in Mulato II reported by Ba *et al.* (2013) (21.9 g/kg) and Ba *et al.* (2014) (19.5 g/kg) were in the same range as levels observed in our experiment.

Tiller counts are a more accurate indicator of seasonal variation in plant condition than yield given non-constant cutting intervals. Rainy season tiller decline may have occurred due to decreased photosynthetic opportunities (cloudy, cool conditions), combined with plant stress from water logging and flooding, and high tiller senescence and competition among aging plants.

The large decline in tiller count at the end of 2011 may explain lower yields in year 2 of the experiment.

4.2 Partial nutrient balances

Negative balances suggest that yields will not be sustainable over time even for the highest input levels used in this experiment. For example, DM yield of 20 Mg/ha per yr with 2.4% N removes 480 kg N/ha per yr in forage mass. Supplying 480 kg N/ha per yr with the cheapest commercial N fertilizer available in Vietnam (urea, 46%N, $0.56 USD/kg in 2013) would require 1,043 kg urea at a total cost of $584 USD/ha per yr. This calculation assumes no losses and application at the crop removal rate, assuming limited soil N supply. Supply of other nutrients in addition to N could be achieved using composted cattle manure if applied in sufficient amounts.

The 24 Mg DM/ha per yr of compost added in the experiment contained approximately 278 kg N, 65 kg P, 163 kg K, 74 kg Mg, and 240 kg Ca. Maximum N release for compost mineralization combined with urea application at removal rate could be sufficient to support a 20 Mg DM/ha per yr yield with assumed losses of 40% of total N applied. Other nutrients removed in harvested biomass with a 20 Mg/ha per yr yield were estimated at 75 kg P/yr, 424 kg K/yr, 70 kg Mg/yr, and 137 kg Ca/yr, based on average plant tissue concentrations determined for the forage. These results suggest that only the supply of Mg and P was adequate while K supply remained insufficient (-261 kg/ha per yr). Yet, a response to added K was not observed in this experiment, suggesting some capacity of the soil to supply K despite low post-experiment soil test levels. Grasses exhibit luxury consumption of K, although yield may remain stable with herbage K concentrations as low as 13 g K/kg DM (Cherney *et al.*, 1998).

Nutrient recycling from decaying above and belowground Mulato II litter could supply additional nutrients. In a grazing system with *Brachiaria humidicola* pastures in Brazil, Boddey *et al.* (2004) discovered litter deposits as high as 170 kg N/ha per yr (30 Mg DM/ha per yr) with low stocking rates. Magnitude of litter contributions is unknown in this experiment, but litter could make a large and important contribution to annual nutrient supply. Further experimentation is necessary in sandy tropical soils to obtain more accurate estimates of nutrient balances.

Reasons reported by farmers for yield persistence (despite negative balances) include nutrient uptake from buffers and neighboring plots, and soil nutrient reserves. *Brachiaria spp.* grasses are deep-rooted, which can help improve soil properties and transfer nutrients from deeper layers (Amézquita *et al.*, 2004). Assessment of the soil fertility status of deeper soil layers was not done for this study. Additionally, rising water tables during the rainy season may bring dissolved nutrients that could be used by actively growing plants and left behind in soil solution after the rainy season (Ponnamperuma, 1984). Furthermore, *Brachiaria spp.* association with N_2 fixing bacteria could supply additional N. Biological N fixation capacity for *Brachiaria spp.* grasses has been identified using ^{15}N labeling, and could supply as much as 20 to 40% of total N uptake (30 to 40 kg N/ha per yr) (Boddey and Victoria, 1986; Reis *et al.*, 2001). A more minor contributor of N is OM. A hectare furrow slice (17-cm deep) contains 2,240,702 kg soil and approximately 11 mg/kg organic-bound N. Approximately 25 kg N/ha per yr could be released from topsoil OM in this study, a small amount compared to the 330 kg N/ha per yr in average crop removal across plots. Atmospheric N deposition could make a small additional contribution on a seasonal basis, perhaps as high as 10 to 15 kg N/ha per yr (Eickhout *et al.*, 2006). Magnitude, and in some cases existence, of these additional contributions is unknown, but could explain the negative partial nutrient balances.

Partial nutrient balances calculated in this experiment did not account for potential nutrient losses that could make balances even more negative. Leaching losses can occur where compost or urea are applied to sandy soils. Water-logged and flooded plots during the rainy season lend favorable conditions for denitrification losses. High rainfall conditions in sandy soils may induce an annual “reset” of nutrients in soil solution, potentially inhibiting treatment response potential in this experiment. Surface applications of urea and manure (as done in experiment year one) furthermore favor losses from volatilization and runoff.

Nitrogen recovery was poor for compost application without urea (Table 16) primarily due to relatively low yields for compost-amended plots. In general, N recoveries were better for higher combined rates of compost and urea. In sandy, nutrient depleted Zimbabwean soils, similar low N recovery was reported for compost (4% recovery rate) applied to corn at 17 Mg DM/ha per yr (0.78% N) (Chikowo *et al.*, 2004). In the study in Zimbabwe, compost combined with 40 kg N/ha per yr ammonium nitrate top-dressed to corn increased N recovery to 8%. Chikowo *et al.* (2004) concluded that low quality composted cattle manure could not supply adequate N and that most N needs would need to be fulfilled by mineral N. Similar relatively low N recoveries were reported by Lynch *et al.* (2004) for perennial forage production with composted dairy manure applied at 12.8 and 25.6 Mg DM/ha per yr in a temperate system (8.9% to 15.1% recovery).

4.3 Soil fertility

Mehlich-3 soil test guidelines for agronomic crops in the subtropics (Florida) suggest medium levels at 26 to 40 mg/kg P, 26 to 40 mg/kg K, and 11 to 23 mg/kg Mg (Kidder *et al.*, 2002). Although local calibration experiments should be conducted, these results suggest the potential for P and K deficiency in our experiment (medium range), while Mg is sufficiently high. Alternative interpretation guidelines for forage crops in temperate U.S. regions suggest that P, K and Mg levels are below optimum for forage crops (optimum range is 30 to 50 mg/kg for P, 100 to 200 mg/kg for K and 120 to 180 mg/kg for Mg) (Beegle, 2013). Variability in soil fertility among farms was high as indicated by large standard deviations for most elements. Pre-experiment farm soil fertility levels were consistent with overall yield differences observed in the experiment (i.e., pre-experiment soil nutrient concentrations were higher for high yielding farms). Furthermore, the interaction of pre-experiment soil fertility with main compost and urea effects for OM and some macro- and micro-nutrients in compost (P, K, Mg, S, Zn, Mn, Cu) suggests that the ability to maintain soil fertility is dependent on the interaction between soil organic and inorganic amendments and existing soil fertility. These observations support the hypothesis that soils are deficient in multiple nutrients, and that compost supplies most soil nutrients but insufficient N, while urea application can address the N shortfall.

Soil OM increase during the experiment also took place in plots that did not receive compost, indicating that litter from above and belowground plant biomass may be accumulating over time. Combination of OM from compost and from Mulato II leaf and root residues probably contributed to higher topsoil OM. Compost may play an important role in maintaining or perhaps increasing soil OM over time.

Soil Mn was most highly correlated with yield and there were possible Mn deficiencies on some farms with low pre-experiment Mn levels (plots at or below 1 mg Mn/kg) that were ameliorated by compost mineralization over time, further supporting the hypothesis that compost addition increases soil fertility status. Overall, compost plays a potentially important role in maintaining OM, preventing acidification, and supplying key macro- and micronutrients for plant uptake. Urea supplies readily available N, which the grass could benefit from once other nutrient limitations had been overcome (with compost application).

4.4 Implications

Land and labor may be more limiting than nutrient availability for forage production in smallholder systems in the study region. Parsons *et al.* (2013) reported 2.4 people/household with SD ± 1.6 in available labor and 0.609 ha with SD ± 0.737 in agricultural land. Backyard landholdings that could be allocated to forage production are often scarce. However, such backyard forage plots could ease pressure on labor resources (Ba *et al.*, 2013; Stür *et al.*, 2013; Khanh *et al.*, 2014). Thus, farmer interest in production of well-managed, small, backyard forage plots could increase in the study region. Stür *et al.* (2013) demonstrated in Vietnam's Central Highlands that farms adopting forage production invested 3 h/d in cattle production labor versus 6.8 h/d for non-adopters. Returns to cattle related-labor were calculated at $0.73/h for adopters and $0.16/h for non-adopters (2005 rates). They concluded that labor was a key reason for adoption of cultivated forage technology, and that labor contributed to observed expansion of cultivated forage areas over time.

An environmental benefit of backyard forage production is decreased pressure on communal grazing lands in the semi-intensive system. Therefore, adoption of backyard forage systems like the one used in our experiment at commune-wide scale could decrease the risk of overgrazing in and near natural areas. Furthermore, grazing shortfalls likely occur during the late dry season and potential for high yields for cultivated forages during this time, especially on irrigated ground, would coincide with periods of peak supplementation demand.

Participating farmers in this experiment owned an average of 0.85 ha of land, 7% (603 m^2) of which was allocated to forage production for cattle diet supplementation. Forage plots were located near the household and cattle shed, which simplified daily management. An average household plot could produce 1.2 Mg forage DM/yr (assumed production of 20 Mg DM/ha per yr), enough to supplement five animals with approximately 241 kg DM/animal/yr. Further assuming 175 kg mean bodyweight (0.7 tropical livestock units, (Jahnke, 1982)) and 2% bodyweight/d in dry matter intake, Mulato II could fulfill approximately 19% of daily dry matter intake (seasonally variable) for each animal. This is a potentially important contribution to supplementary green fodder, the implications of which merit further investigation with ration balancing software and economic models. Expansion of productive areas could further increase available DM per animal and resulting production potential. For example, expansion to 0.1 ha would increase supplementation potential to approximately 31% of daily dry matter intake requirements.

4.5 Cattle manure availability, fertilizer equivalence and management

If we assume 700 kg manure DM/animal per yr (1,000 kg/tropical livestock unit per year), a herd size of 5 animals, and 525 kg available manure DM/animal per yr (6 hours grazing/day removed), the amount of cattle manure available amounts to 2,625 kg manure DM/yr, and 26 kg N/yr. Assuming no N losses, composted manure could be applied at or near farmer-desired rates for a backyard 0.1-ha plot (up to 263 kg N/ha per yr). However, compost would not be available for application to other crops. A composted manure shortfall would arise, because most farmers apply compost to rice, cassava and peanut production lands. Two farmers reported compost purchases from neighboring farms at 300,000 VND/ox cart load (\$14.29 USD/~0.5 m^3 or 500 kg dry manure). Thus, composted manure cost is approximately 600 VND/kg dry manure (2.86¢/kg) or 60,000 VND/kg N (\$2.86 USD/kg N), which is more expensive per N unit than urea (11,700 VND/kg 46% urea or 25,435 VND/kg N, \$1.21 USD/kg N). Thus, investment in urea is more cost effective for N supply than composted cattle manure based on 2013 rates. However, results of this experiment have demonstrated that addition of urea alone cannot increase yields and co-application of both nutrient sources is needed to increase yields, consistent with findings of Zingore *et al.* (2008).

A whole farm nutrient cycling efficiency study in Kenya (similar manure management system) reported that just 27% of nitrogen excreted by cattle was returned to the soil (Castellanos-Navarrete *et al.*, 2014). Sound manure management could increase nutrient concentration of composted manure and increase manure N cycling efficiencies (Rufino *et al.*, 2007; Castellanos-

Navarrete *et al.*, 2014). For example, improved manure storage techniques can reduce N losses due to leaching by 25% (Markewich *et al.*, 2012). Controlled forage harvest systems with manual cutting like the one used in our experiment have demonstrated lower nitrate leaching losses in sandy soils relative to grazing (Wachendorf *et al.*, 2004). Markewich *et al.* (2010) discovered that mineral N content in manure declines when storage times exceed 30 d. Short storage times between manure production and application on agricultural lands, combined with covering of compost piles, installing cement floors for manure storage, and collecting leachate could increase available manure N. Better management could decrease nutrient losses, ensure higher nutrient concentrations in available composted manure, and enhance farm nutrient cycling efficiencies.

5. Conclusions

Our study suggests that application of urea to *Brachiaria* Cv. Mulato II is only effective when combined with composted cattle manure, reflecting nutrient deficiencies of the sandy soils in south-central coastal Vietnam. We conclude that proper compost and fertilizer management can increase crop yield, but relatively high application rates are needed for sustainable growth of Vietnamese smallholder beef systems on sandy soils.

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APPENDIX 1: COMPLEMENTARY SOIL FERTILITY DATA

Table A1 Pre- and post-experiment soil fertility means at the 15 to 25-cm depth for compost x urea treatment combinations. Elements are Mehlich-3 extractable nutrients. Pre-experiment data are included as a treatment baseline. Means calculated in each cell are an average of 6 farms. The only pair that differed significantly based on a paired one-tailed t test, using the Bonferroni-adjusted P value cutoff of 0.003, is highlighted in gray.

	Compost Treatment (Mg DM/ha per yr) / Urea Treatment (kg N/ha per yr)														
Parameter	**0**	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**
	0	**0**	**0**	**0**	**0**	**60**	**60**	**60**	**60**	**60**	**120**	**120**	**120**	**120**	**120**
pre-pH (1:1 soil:water)	5.37	5.98	5.63	5.87	5.68	5.52	5.27	5.83	5.83	5.63	5.72	5.50	5.82	5.75	5.45
post-pH (1:1 soil:water)	5.05	5.30	5.47	5.48	5.67	5.58	5.05	5.48	5.37	5.50	5.18	5.32	5.32	5.10	5.32
pre-CEC (cmol$_+$/100 g)	1.19	1.16	1.19	1.22	1.53	1.52	1.20	1.40	1.29	1.43	1.33	1.17	1.35	1.28	1.23
post-CEC (cmol$_+$/100 g)	0.922	1.10	1.13	1.16	1.47	1.69	1.05	1.26	1.26	1.26	1.14	1.04	1.31	1.44	1.25
pre-OM (g/kg)	4.07	2.58	2.87	3.05	4.15	4.68	3.28	3.35	3.13	3.87	3.98	3.47	3.15	4.22	3.15
post-OM (g/kg)	6.82	5.5	5.97	6.65	7.23	6.62	7.12	6.13	6.1	7.48	4.8	4.98	5.53	6.32	5.58
pre-S (mg/kg)	9.67	8.00	9.33	7.50	9.50	9.67	9.33	8.67	8.67	10.30	9.83	9.17	8.17	8.50	9.00
post-S (mg/kg)	8.67	6.83	7.17	7.33	7.67	6.67	8.50	7.00	7.67	7.50	8.33	7.33	7.67	7.67	7.33
pre-P (mg/kg)	36.5	37.7	36.5	27.2	39.0	50.8	33.2	46.2	34.2	39.7	36.2	37.3	42.8	29.3	37.3
post-P (mg/kg)	25.3	18.5	24.2	23.5	36.5	29.0	27.7	26.5	22.0	24.2	23.2	20.7	25.3	17.0	24.2
pre-Ca (mg/kg)	165	159	165	178	207	198	170	193	159	201	174	162	179	165	150
post-Ca (mg/kg)	119	156	154	163	220	181	141	168	185	174	136	141	187	176	169
pre-Mg (mg/kg)	25.8	22.0	23.5	22.0	30.2	31.8	22.8	31.3	33.2	29.2	34.3	23.5	30.7	27.7	34.2
post-Mg (mg/kg)	20.8	20.2	23.8	24.8	26.7	24.0	21.7	23.5	21.5	26.5	20.0	19.7	25.8	16.2	30.5
pre-K (mg/kg)	24.3	35.5	32.7	23.2	47.3	64.8	25.7	29.5	48.3	32.5	26.5	29.5	38.7	33.5	38.2
post-K (mg/kg)	12.3	10.7	10.8	11.8	10.2	16.0	12.2	12.0	11.0	12.5	11.0	11.5	11.8	10.7	12.0
pre-Na (mg/kg)	21.3	20.7	20.5	18.8	28.8	21.2	20.3	22.5	21.3	23.7	24.2	20.8	24.5	32.5	22.7
post-Na (mg/kg)	28.2	29.7	29.3	25.0	27.5	42.3	30.5	28.2	28.0	32.0	26.3	32.0	28.8	28.3	27.8
pre-B (mg/kg)	0.135	0.170	0.228	0.208	0.243	0.203	0.135	0.148	0.100	0.262	0.230	0.075	0.203	0.127	0.138
post-B (mg/kg)	0.262	0.115	0.125	0.090	0.170	0.290	0.223	0.100	0.207	0.128	0.300	0.235	0.143	0.183	0.313
pre-Fe (mg/kg)	132	123	116	97.8	116	153	106	127	115	132	100	128	107	109	114
post-Fe (mg/kg)	138	112	120	132	148	154	125	135	127	125	108	122	152	120	121

Parameter	Compost Treatment (Mg DM/ha per yr) / Urea Treatment (kg N/ha per yr)														
	0	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**
	0	**0**	**0**	**0**	**0**	**60**	**60**	**60**	**60**	**60**	**120**	**120**	**120**	**120**	**120**
pre-Mn (mg/kg)	8.50	5.83	5.17	8.17	8.17	10.5	6.17	5.67	8.00	6.00	6.33	6.67	8.83	11.5	5.33
post-Mn (mg/kg)	6.50	7.00	10.0	8.00	13.7	8.00	9.67	7.00	11.0	6.17	6.17	5.33	9.33	7.50	6.17
pre-Cu (mg/kg)	1.74	1.11	1.19	1.24	1.76	1.45	1.20	1.50	1.69	1.50	2.05	1.94	1.17	2.39	1.56
post-Cu (mg/kg)	1.81	1.65	2.02	1.67	1.73	1.73	1.74	1.59	1.44	2.05	1.99	1.69	2.24	2.02	1.68
pre-Zn (mg/kg)	2.45	1.45	2.06	3.20	3.14	2.72	1.88	1.64	1.76	2.11	3.69	2.09	2.05	10.60	1.61
post-Zn (mg/kg)	1.37	2.12	2.17	5.97	3.53	4.13	1.84	1.59	1.89	2.42	3.57	1.98	3.89	2.25	1.60
pre-Al (mg/kg)	293	235	306	244	273	285	289	339	246	267	369	292	249	225	265
post-Al (mg/kg)	250	192	245	257	254	221	267	248	230	202	313	210	245	230	242
pre-NO_3-N (mg/kg)	3.93	2.40	2.40	2.67	5.10	3.87	3.15	1.90	3.32	2.68	1.67	2.78	3.48	4.57	2.45
post-NO_3-N (mg/kg)	1.37	1.33	0.77	1.25	0.400	0.450	1.87	0.533	1.48	0.650	0.850	0.433	1.98	2.57	1.12
pre-NH_4-N (mg/kg)	8.90	6.13	8.30	6.73	7.60	8.88	8.57	8.65	8.72	8.17	9.73	8.25	7.72	6.70	7.77
post-NH_4-N (mg/kg)	13.5	9.52	9.82	10.2	10.1	10.2	12.4	9.92	10.6	10.7	11.0	10.4	13.5	10.8	10.6

Table A2 Treatment P values for the difference between post-experiment soil fertility parameters and pre-experiment parameters in the 15 to 25-cm stratum. Each row represents a single model. Models include block as a random effect. Elemental analyses are for Mehlich-3 extractable elements. Significant effects ($P \leq 0.05$) are highlighted in gray.

Parameter	Compost	Urea	Compost x urea
Total Exchange Capacity (meq/100 g)	0.9317	0.8727	0.8756
pH (1:1 soil:water)	0.0269	0.1749	0.0595
Organic Matter (g/kg)	0.1604	0.0036	0.7973
S (mg/kg)	0.3240	0.4647	0.7185
P (mg/kg)	0.4661	0.2771	0.4179
Ca (mg/kg)	0.4523	0.8386	0.8740
Mg (mg/kg)	0.3694	0.0898	0.6977
K (mg/kg)	0.7658	0.4829	0.0413
Na (mg/kg)	0.2887	0.1182	0.4206
B (mg/kg)	0.2849	0.1422	0.5266
Fe (mg/kg)	0.3824	0.6983	0.1219
Mn (mg/kg)	0.2557	0.2068	0.4542
Cu (mg/kg)	0.1078	0.4458	0.1693
Zn (mg/kg)	0.7921	0.2485	0.0816
Al (mg/kg)	0.0189	0.2622	0.1371
NO_3-N (mg/kg)	0.4310	0.4914	0.1622
NH_4-N (mg/kg)	0.9296	0.3301	0.0985

Table A3 P values for treatment effects on soil chemical properties in 0 to 15-cm and 15 to 25-cm strata for models containing fixed effects of compost (C), urea (U), compost x urea (C x U), and pre-experiment soil fertility measurement for each parameter sampled at 15 to 25-cm depth (PSF), with block as a random effect. Each row represents a single model for each soil depth. Significant values at p≤0.05 are highlighted in gray.

Parameter	C	U	C x U	PSF	C	U	C x U	PSF
	0 to 15-cm depth				15 to 25-cm depth			
CEC ($cmol_+$/100 g)	0.2145	0.6271	0.3142	0.0797	0.6827	0.5997	0.4332	0.0363
pH (1:1 soil:water)	0.0071	0.1196	0.0705	<0.0001	0.0460	0.1071	0.0856	<0.0001
Field pH	0.1974	0.4280	0.2340	0.0132	0.0331	0.6814	0.5159	0.3117
OM (g/kg)	0.0087	0.6440	0.0505	<0.0001	0.2699	0.0035	0.7938	<0.0001
S (mg/kg)	0.2249	0.9163	0.0720	0.2507	0.8146	0.7345	0.1171	0.0547
P (mg/kg)	0.6723	0.5556	0.3621	0.0157	0.6537	0.3633	0.4554	0.0001
Ca (mg/kg)	<0.0001	0.4688	0.0147	0.0718	0.1873	0.9323	0.6124	0.2071
Mg (mg/kg)	0.0015	0.2469	0.2219	0.0540	0.0542	0.4886	0.4991	0.0006
K (mg/kg)	0.1703	0.3593	0.7202	0.0301	0.2833	0.1041	0.3959	0.3572
Na (mg/kg)	0.0788	0.6333	0.8865	0.0446	0.1636	0.0246	0.0201	0.3319
B (mg/kg)	0.0954	0.4529	0.4883	0.9517	0.0788	0.2146	0.8916	0.5975
Fe (mg/kg)	0.9956	0.4733	0.2342	0.0020	0.4978	0.9109	0.1584	<0.0001
Mn (mg/kg)	0.0002	0.4951	0.6742	0.9859	0.5170	0.2015	0.3686	0.0002
Cu (mg/kg)	0.5569	0.3744	0.5829	0.0343	0.3963	0.8292	0.4546	0.0003
Zn (mg/kg)	0.8383	0.6481	0.2006	0.1849	0.9202	0.5418	0.0771	0.0046
Al (mg/kg)	0.6086	0.4611	0.6980	<0.0001	0.1240	0.2865	0.1456	<0.0001
NO_3-N (mg/kg)	0.5608	0.2398	0.2026	0.1510	0.1936	0.4103	0.2641	0.0885
NH_4-N (mg/kg)	0.1017	0.6409	0.1306	0.0155	0.9530	0.5630	0.0386	0.0068

CHAPTER 2: IMPACT OF FERTILIZATION WITH UREA AND COMPOSTED CATTLE MANURE ON *BRACHIARIA* CV. MULATO II NUTRITIVE VALUE IN SOUTH-CENTRAL VIETNAM

Abstract

To obtain sustainable growth of Vietnamese smallholder beef systems, production of improved forages in backyard plots is essential. Our objectives were to evaluate the effects of composted cattle manure and mineral nitrogen (urea) on *Brachiaria* Cv. Mulato II (*B. ruziziensis* x *B. decumbens* x *B. brizantha*) forage nutritive value in Cát Trinh Commune, Bình Định Province. The study was conducted from late 2010 to early 2013 using a randomized complete block design on six farms (blocks), with five rates of composted cattle manure (0, 4, 8, 12, and 24 Mg DM/ha per yr) and three urea rates (0, 60, and 120 kg N/ha per yr) in a factorial design. Treatments were split-applied at 2-mo intervals, and grass was harvested at approximately monthly intervals. Forage nutritive value was measured for two harvest periods in September 2011 and December 2012. Compost application increased ash in both harvest periods analyzed together ($P=0.0072$), and K ($P=0.0231$) and Mg ($P=0.0010$) for one of the two harvests, suggesting seasonal differences. Urea increased the nitrogen nutrition index ($P=0.0065$) for both harvests and increased lignin for one period. Harvest interval, climatic factors, and plants count in experimental plots influenced DM concentration of fresh forage biomass more than compost and urea addition. Urea increased nutrient yield for all forage nutritive value parameters measured. Fertilization of tropical forages such as Brachiaria Cv. Mulato II with urea and compost impacts several nutritive value parameters, but other climatic and management factors such as cutting interval are likely more important determinants of forage nutritive value for cattle production.

1. Introduction

Smallholder crop-livestock farms (≤ 5 head cattle/household) in south-central coastal Vietnam contribute to beef supply for urban areas (Parsons *et al.*, 2013). Semi-intensive (grazing and stall feeding) and extensive (grazing of communal land) cattle management systems are dominant in the region. Supplementary stall feeding with a basal diet of rice straw, peanut straw, and cultivated forage (e.g., *Pennisetum purpureum*) or cut-and-carry native grasses and legumes from private and communal land complements supervised or unsupervised grazing on communal (and private) lands (Parsons *et al.*, 2013). Many farmers have initiated a transition toward semi-intensive management to increase animal productivity (daily gain) using supplementary forages (native grasses and legumes and cultivated improved forages) and reducing energy expenditures incurred from walking to open grazing areas. Key to this transition is cultivation of high yielding, high nutritive value forages that are well-adapted to local climatic and soil conditions (Ba *et al.*, 2013; Parsons *et al.*, 2013; Stür *et al.*, 2013; Ba *et al.*, 2014). Farmers also benefit from reduced labor requirements for cattle production when forages are cultivated near the household (Stür *et al.*, 2013; Khanh *et al.*, 2014).

Data supporting forage management decisions in sandy, tropical, rain-fed systems such as those located in the south-central Vietnam coast are inadequate (Devendra, 2002; Stür *et al.*, 2002). We describe in a companion paper based on a multi-year field experiment in Vietnam that combined application of composted cattle manure and urea was required to increase *Brachiaria* Cv. Mulatio II yields (McRoberts, Chapter 1). Nutrient deficiencies in sandy soils could be at least partially overcome by a combination of macro-and micronutrients in compost (including N) and N in urea, and are necessary to maintain soil fertility and drive forage productivity over time.

Little is known about the impact of fertility management on forage nutritive value in tropical crop-livestock systems in general and in Vietnam, specifically. Tropical forage growth conditions like those found in the study region of Vietnam present unique management challenges when farmer objective is to harvest high nutritive value (rather than yield), although these objectives are often complementary and create decision tradeoffs for farmers.

Ephemeral changes in forage nutritive value are often dependent on climate-related factors with rapid day-to-day and interdiurnal variation. These effects are typically smaller in the tropics due to lower fluctuation in photoperiod and temperature relative to temperate regions (Van Soest, 1994). In the tropics, high temperatures and relatively constant day length drive an inconsistent relationship between digestibility and fiber, due to the poor relationship between cellulose and lignin (Buxton and Fales, 1994; Van Soest, 1994). Thus, harvest management decisions are complicated, because fiber concentration is less effective in predicting animal response in the tropics (Van Soest, 1994). Furthermore, high temperatures and high prevalence of C_4 grasses promote high concentration of cell wall components, lignification, and poor digestibility (Van Soest, 1994). Water availability is more important in the tropics and seasonal precipitation patterns in rain-fed forage systems affect yield and forage nutritive value (Buxton and Fales, 1994; Van Soest, 1994); drought can improve forage digestibility at the expense of yield due to slower plant development and maturation, while excess rainfall tends to cause lignification (Van Soest, 1994; Van Soest, 1996).

Nitrogen fertilization is essential to obtain higher yields, and can increase crude protein concentrations in forage as well (Peyraud and Astigarraga, 1998). Nitrogen-fertilized grasses also tend to grow faster, generating a more lignified plant faster due to more rapid plant growth and maturity (Van Soest, 1994; Van Soest, 1996). Plant available N is key to short-term high yields of perennial forage grasses. Slower release forms of N and other macro- and micronutrients from mineralization of composted cattle manure and crop residues could be important to supply other nutrients critical to sustained growth and high nutritive value (Palm *et al.*, 1997).

The objective of this study was to assess the effects of urea and composted cattle manure on forage nutritive value. The effects of urea and composted cattle manure on yield and soil properties are reported in a companion paper (McRoberts, Chapter 1).

2. Methods

2.1 Cát Trinh Commune characteristics

The experiment was undertaken from 2010 to 2013 in Cát Trinh Commune, Phù Cát District, Bình Định Province in south-central coastal Vietnam. The district capital of Ngô Mây (14°0′2″ N, 109°2′38″ E) borders Cát Trinh Commune to the southwest. Sandy soils in the commune belong to the Arenosols Group. Annual rainfall in the region is about 1,200 mm, with over 70% falling during the rainy season (September to December). The range in average monthly temperature is from 23 to 31°C. Farms were selected in regions where the Australian Centre for International Agricultural Research-funded Project SMCN/2007/109, entitled, "Sustainable and Profitable Crop and Livestock Systems for South-central Coastal Vietnam", was taking place. Project presence simplified access to commune farms, including permission requirements with local and regional authorities, and permitted adequate experiment tracking due to relationships with rural development professionals and extension educators operating in the commune. This experiment responded directly to problems identified by local farmers and revealed during baseline project surveys (Hoa, 2009; Parsons *et al.*, 2013).

2.2 Farm selection and experiment preparation

Commune extension educators assisted with selection of six farms according to the following criteria: (1) non-participants in previous Australian Centre for International Agricultural

Research initiatives, (2) cattle owners, (3) interest in participation, (4) availability of a well-drained, level parcel near the household (13 m x 13 m or equivalent), and (5) access to irrigation. Recent field histories included: (1) *Pennisetum purpureum* Schumach. cultivation, (2) vacant with household waste and ash accumulation, (3) cassava and eggplant production, (4) cassava production, (5) peanut production, and (6) vacant lot. A range of backyard sandy soils in Vietnam and potentially in other tropical regions were represented in initial soil fertility conditions. Pre-experiment (2010) soil samples at a depth of 15-25 cm indicated low organic matter (0.35% with SD ± 0.207%), low nutrient concentrations (Mehlich 3-extracted P = 38 mg/kg with SD ± 27.3 mg/kg; Mehlich 3-extracted K = 36 mg/kg ± 36.2), and acid soils (pH (1:1 soil:water) = 5.67 ± 0.658). Soil analyses in the experiment are reported in McRoberts (Chapter 1).

Farmers and researchers prepared experimental areas using animal traction and handheld hoes in July 2010 according to farmer practice. Weeding was done using handheld hoes as needed throughout the experiment (typically one to two times per month). A woven wire fence (1.2-m high) was installed around each area to inhibit animal entry. No pesticides were applied during the experiment.

Drought tolerance, adaptation to acid soils, and high nutritive value relative to other C_4 forages made *Brachiaria* Cv. Mulato II (*B. ruziziensis* x *B. decumbens* x *B. brizantha*) a favorable choice for the agronomic conditions in Cát Trinh Commune (Argel *et al.*, 2007; Inyang *et al.*, 2010; Vendramini *et al.*, 2012). A high density (> 500 plants/m^2) covered Mulato II seedbed was established at a participating farm in August 2010. Young plants were transplanted into experimental areas approximately four weeks after seedbed establishment (15 to 30-cm maximum height). Starter fertilizer (20-20-15; N-P_2O_5-K_2O) was applied at 175 kg/ha before transplanting in September 2010, and a second time after transplanting in October 2010. Mulato

II plant establishment density in each 2 x 2-m plot was 20 plants, derived from 50-cm row spacing and 40-cm plant spacing. Replanting was completed during the first two months after establishment (September and October 2010) to obtain desired density (50,000 plants/ha). One-meter buffer strips and outer borders separated plots and contained single row of Mulato II with 40-cm spacing between plants.

2.3 Experimental design

Fifteen treatments were derived from factorial combination of five compost rates (0, 4, 8, 12, and 24 Mg DM/ha per yr) and three urea rates (0, 60, 120 kg N/ha per yr) in a randomized complete block design on six farms (blocks). A sixteenth treatment added 80 kg K_2O/ha per yr to the highest compost rate in 2011, and also included the highest urea rate from January 2012 onward. Buffer strips received 4 Mg DM/ha per yr compost.

Farmer practice dictated compost preparation, including daily removal of manure and rice straw refusals from stalls and transfer to an uncovered pile for approximately 45 d. The pile was turned at least two times prior to use. A single participating farm provided all compost to ensure treatment consistency. Analysis of pre-experiment compost samples (50% DM, 1% N) determined field application rates to achieve desired compost N rates (0, 40, 80, 120, and 240 kg N/ha per yr). Actual N rates differed somewhat due to variation in compost DM and N concentrations, and are reported along with full compost treatment composition in McRoberts (Chapter 1). Treatments were selected to balance for Mulato II N requirement (240 kg N/ha per yr) with the highest compost treatment as suggested by J. Corfield (personal communication, July, 2010).

Annual treatments were divided into six parts and applied at approximately 2-mo intervals immediately following each harvest. During the first year, treatments were surface applied, while second year treatments were incorporated near the soil surface (top 0 – 15-cm) with handheld hoes to reduce potential for nutrient transfer across plots and to prevent nutrient loss. In November 2010 (two months after transplanting), all plants were cut uniformly at 25-cm above ground level with a handheld sickle to encourage vertical tiller development. Initial compost and urea treatments were applied at that time.

2.4 Sampling protocols

2.4.1 Forage samples

Short cutting height (5-cm) and harvest intervals (2-wk) increase DM yield, but negatively impact persistence of Mulato II (Inyang *et al.*, 2010; Vendramini *et al.*, 2014). Thus, a 30-d cutting interval and 15-cm cutting height were selected to achieve high yield, persistence, and high forage nutritive value. Plots were harvested (24 total harvest events) at approximately monthly intervals (36 d with SD ± 8.6 d) using handheld sickles beginning in January 2011. Cutting height increased from 15-cm to approximately 25-cm over the course of the experiment as crowns thickened. All plots could not be harvested on the same day. Consequently, farms were harvested within two to four days and in the same order for each harvest event. Cutting intervals were longer and more variable in 2011 (39 d with SD ± 12.3 d) than for the rest of the experiment (33 d with SD ± 3.3 d), due to flooding in the study area in October 2011 that prevented harvest.

All fresh forage biomass in each plot was collected and weighed. Subsamples (200 to 300 g) were collected every other harvest event to measure forage nutritive value, while smaller subsamples (20 to 50 g) were collected to determine DM concentration during alternate harvests. Dry matter concentration was determined at a nearby research station by drying samples to stable weight at 60°C in a forced-air oven. Subsamples for forage nutritive value were ground to pass a 4-mm screen in a Retsch cutting mill (Haan, Germany) at Huế University of Agriculture and

Forestry, and divided using a sample separator. Only samples that were not visibly damaged by heat or water during the post-harvest transportation and storage process were reground to pass a 1-mm screen in a UDY Cyclone Mill (UDY Corp., Fort Collins, CO) at Cornell University. Dairyland Laboratories, Inc. (Arcadia, WI) determined nutritive value for samples collected in September 2011 and December 2012 using near infrared spectroscopy (Marten *et al.*, 1989) with a Foss model 5000 (Foss-NIR System, Silver Spring, MD). Nutritive value parameters included crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, NDF digestibility at 30 h, in vitro DM digestibility at 30 h, acid detergent insoluble crude protein, neutral detergent insoluble crude protein, soluble protein, fat, ash, Ca, P, Mg, K, S, sugar, non-fiber carbohydrates, digestible DM, digestion rate, and total fatty acids.

Nutrient yields were calculated by multiplying DM yield by nutrient concentration in September 2011 and December 2012 harvests. Nitrogen nutrition index (NNI) was calculated as $NNI = N_a/N_c$ and $N_c = aW^{-b}$, where N_a is actual N concentration, N_c is critical N concentration, W is DM yield (Mg/ha), and a and b are species specific constants for C_4 perennial grasses (3.6 and 0.34, respectively) (Lemaire *et al.*, 2008; Alderman *et al.*, 2011). Luxury N consumption was assumed for NNI values > 1 (Lemaire *et al.*, 2008).

2.5 Weather

September 2011 samples were collected at the beginning of the rainy season before the onset of heavy rainfall. December 2012 samples were collected at the beginning of the dry season. Total rainfall in September 2011 harvest (49-d cutting interval) was higher than in December 2012 (32.5-d cutting interval). Mean temperature was 3ºC higher in the growth period associated with the September 2011 harvest. Most rainfall occurred during the September to December rainy season in 2011 (76%) and 2012 (55%). Dry season rainfall was low and consistent with historical patterns. Temperatures were within normal ranges.

2.6 Statistical methods

Mixed model procedures analyzed results using the standard least squares personality and restricted maximum likelihood method of JMP PRO 11.2.0 (SAS Institute Inc., 2013). Models included fixed effects of compost rate, urea rate, and their interaction, and block (farm) as a random effect. Harvest period was included as a covariate when September 2011 and December 2012 data were analyzed together. These data were also analyzed separately, and P values are presented in Appendix 1. Nutritive value treatment means for the compost x urea interaction are also available (Appendix 2). Mean differences among treatments were declared at $P \leq 0.05$ using Tukey's statistic to control the family-wise error rate for multiple comparisons. Data normality was checked via visual assessment of distribution of model residuals.

Dry matter concentration in fresh forage biomass was measured during all 24 harvest events, and candidate covariates in DM model selection included harvest interval, pre-experiment soil fertility, rainfall, and mean temperature, as well as block and block x treatment (plot) as random effects to account for non-independence of observations within farms and within plots over time. Compost and compost x urea interaction were not significant in nutrient yield models and were excluded from analyses completed for September 2011 and December 2012 harvests. Thus, nutrient yield models contained fixed effects of urea and harvest period (September 2011 and December 2012), and block as a random effect. Nutrient yield responses were log- or square root-transformed.

3. Results and discussion

3.1 Forage nutritive value

Plant tissue samples for two representative harvest periods (n=96 for each period) indicated high nutritive value as forage for cattle (Table 1). Forage nutritive value was consistent with results reported in the literature (Argel *et al*., 2007; Inyang *et al*., 2010; Ba *et al*., 2013; Ba *et al*., 2014) for Mulato II. Nutritive value in September 2011 and December 2012 harvests was similar, but notable differences were higher sugar and non-fiber carbohydrate levels in September 2011 samples and higher crude protein in December 2012 samples. Cooler, wetter, cloudier conditions near December 2012 harvest relative to the September 2011 harvest could explain these differences (Van Soest, 1996).

Nutritive value for the 16th treatment containing potassium did not differ from any other treatments (results not shown). Compost and urea application affected few nutritive value parameters, and results were inconsistent for the September 2011 and December 2012 harvest periods (Table 2; Appendix 1).

Compost addition decreased DM concentration in pooled analysis of September 2011 and December 2012 samples (n=180) (Figure 1). Dry matter in the highest compost application rate was lower than 8 Mg DM/ha per yr and plots not receiving compost. Compost application increased ash concentration (%DM) (Figure 1). The highest ash concentration was measured when compost was applied at 24 Mg/ha per yr, which was significantly higher than plots not receiving compost and those receiving 12 Mg DM/ha per yr and 8 Mg DM/ha per yr. Compost also increased K concentration in September 2011 samples and Mg concentration in December 2012 samples, with K and Mg concentrations in the highest compost rate higher than without compost (Figure 1, Table 2). Compost application tended to increase P in September 2011 samples, with P concentration when compost was applied at 24 Mg/ha per year higher than plots not receiving compost (Figure 1, Table 2). Compost tended to decrease sugar in September 2011 samples, but mean differences were not detected (Figure 1, Table 2).

Table 1 Harvest period characterization and nutritive value parameters for Mulato II forage harvests in September 2011 and December 2012 on six farms (16 plots/farm).

Variable	**Sept 2011 (n=96)**		**Dec 2012 (n=96)**	
	Mean	**SD**	**Mean**	**SD**
n	96		96	
Harvest interval (d)	49		32.5	
Rainfall (mm)	112.6		48.1	
Mean temperature (ºC)	29.7		26.3	
Dry matter (DM) yield (Mg/ha)	3.34	2.15	1.14	0.771
Dry matter (%)	22.1	3.03	18.4	2.19
Crude protein (% DM)	13.1	2.04	17.0	3.48
Nitrogen nutrition index[a]	0.811	0.176	0.745	0.217
Acid detergent fiber (% DM)	35.8	3.00	34.0	2.33
Neutral detergent fiber (% DM)	62.1	3.88	63.0	3.18
Lignin (% DM)	4.61	0.313	4.16	0.447
Neutral detergent fiber digestibility at 30 h (% NDF)	59.3	2.39	63.2	1.69
In vitro dry matter digestibility at 30 h (% DM)	74.6	2.94	76.8	2.00
Acid detergent insoluble crude protein (% CP)	0.579	0.115	0.922	0.141
Neutral detergent insoluble crude protein (% CP)	1.95	0.350	4.71	0.610
Soluble protein (% CP)	30.9	5.12	31.9	4.62
Fat (% DM)	3.43	0.281	3.58	0.255
Ash (% DM)	11.0	0.878	11.7	0.811
Ca (% DM)	0.601	0.109	0.771	0.0887
P (% DM)	0.372	0.0308	0.380	0.0395
Mg (% DM)	0.285	0.0345	0.415	0.0393
K (% DM)	2.12	0.510	2.12	0.375
S (% DM)	0.242	0.0268	0.282	0.0335
Sugar (% DM)	9.35	0.921	4.92	0.486
Non-fiber carbohydrates (% DM)	11.5	2.73	6.33	1.08
Digestible dry matter (% DM)	61.1	2.33	62.4	1.81
Kd rate (Digestion rate) (%/h)	3.92	0.316	4.20	0.363
Total fatty acids (% DM)	1.95	0.444	1.83	0.268

[a] Nitrogen nutrition index (NNI) was calculated as $NNI = N_a/N_c$ and $N_c = aW^{-b}$, where N_a is actual N concentration, N_c is critical N concentration, W is DM yield (Mg/ha), and a and b are species specific constants for C_4 perennial grasses (3.6 and 0.34, respectively) (Lemaire *et al.*, 2008; Alderman *et al.*, 2011). Luxury N consumption was assumed for NNI values > 1 (Lemaire *et al.*, 2008).

Table 2 Selected main effect P values for Mulato II forage nutritive value responses in separate mixed models for September 2011 and December 2012 samples (n=90 for each period). Models contained fixed effects of compost, urea, and compost x urea interaction, and block as a random effect. Significant effects are highlighted in gray.

Parameter	September 2011			December 2012		
	Compost	Urea	Compost x urea	Compost	Urea	Compost x urea
Dry matter (%)	0.0095	0.2663	0.8596	0.3898	0.9211	0.9910
Nitrogen nutrition index	0.2106	0.0051	0.6658	0.7029	0.2277	0.1867
Lignin (% DM)	0.6133	0.0469	0.6304	0.4513	0.3931	0.3557
Ash (% DM)	0.2562	0.3903	0.4520	0.0385	0.4924	0.5476
P (% DM)	0.0583	0.8089	0.9528	0.2210	0.6370	0.6447
Mg (% DM)	0.1355	0.1410	0.6443	0.0010	0.1734	0.5611
K (% DM)	0.0231	0.8744	0.3860	0.0831	0.1881	0.5453
Sugar (% DM)	0.0577	0.3467	0.4328	0.4104	0.5762	0.5334
Non-fiber carbohydrates (% DM)	0.1540	0.1919	0.7959	0.0497	0.5853	0.8705

The effect of compost on ash, K, and Mg concentrations is consistent with work by González *et al.* (2011) that reported a positive K response to cattle manure. These effects could be important for formulation of cattle rations. Most farmers in south-central coastal Vietnam supply salt, but only about 40% of farmers in the study area provide supplemental minerals (Parsons *et al.*, 2013). Minerals must be obtained from water, herbage intake, and soil ingested while grazing. Transition to semi-intensive operations with less grazing limits opportunity for mineral intake, making mineral supplementation even more important. Mineral concentration of Mulato II could be adequate to fulfill cattle requirements (Van Soest, 1994), but should be verified during ration formulation. Compost also impacted non-fiber carbohydrates in December 2012 samples, but results were inconsistent and mean differences were not detected.

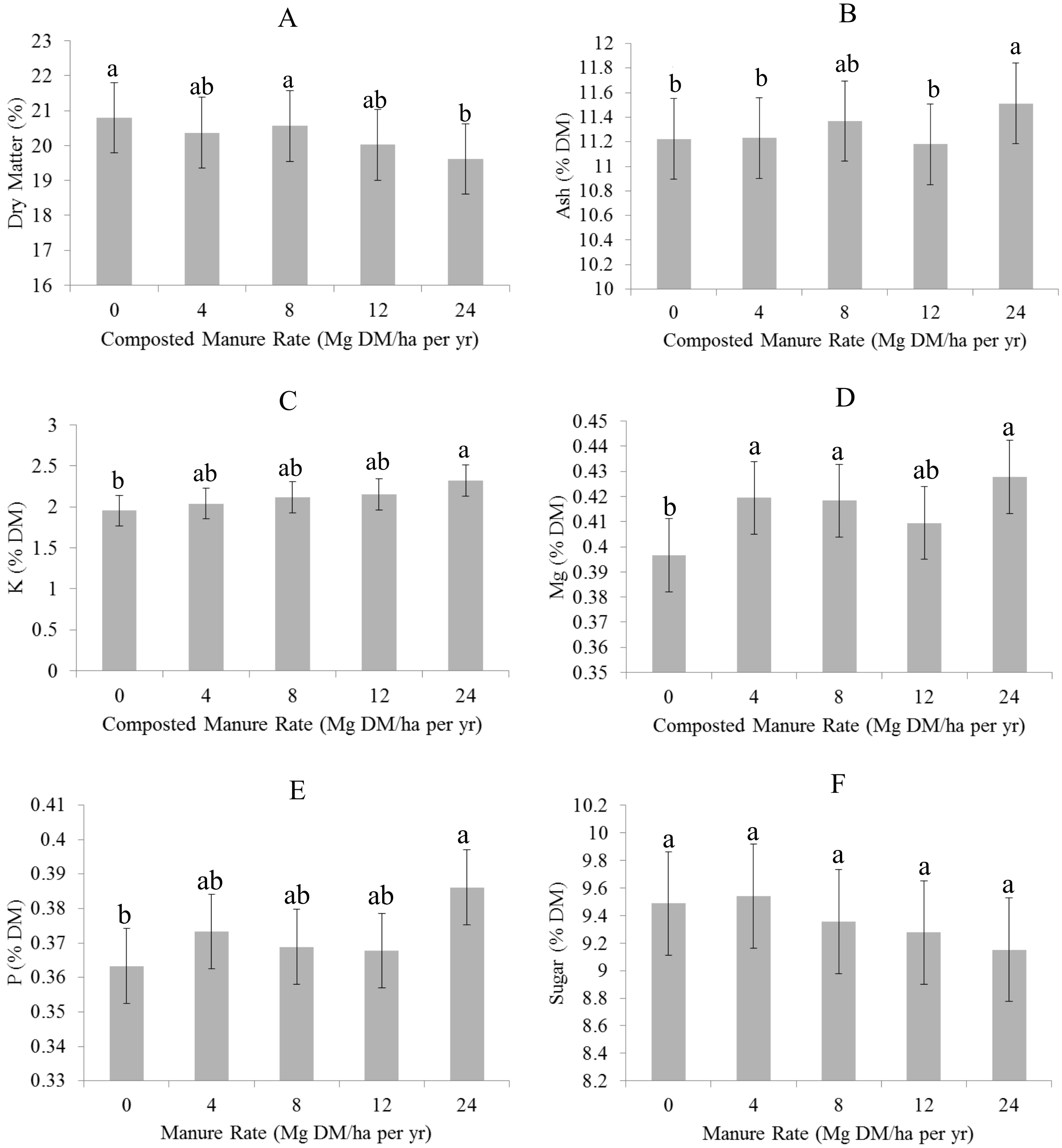

Figure 1 Least square means for effect of compost on *Brachiaria* Cv. Mulato II dry matter (A, n=180, P=0.0060) and ash (%DM) (B, P=0.0072) in September 2011 and December 2012 samples, K (%DM) in September 2011 samples (C, n=90, P=0.0231), Mg (%DM) in December 2012 samples (D, n=90, P=0.0010), P in September 2011 samples (E, n=90, P=0.0583), and sugar in September 2011 samples (F, n=90, P=0.0577). Error bars are 1 SE of the mean. Bars connected by the same letter in each bar graph are not significantly different.

Urea increased NNI values in pooled September 2011 and December 2012 samples, with NNI in plots receiving the highest urea application rate (0.819) higher than the intermediate rate and without urea (Figure 2, Appendix 1). Nitrogen nutrition index means were < 1, indicating that N critical levels were not reached. The urea treatment did not affect crude protein concentration in harvested biomass as suggested by response to inorganic N fertilization in experiments with other species (Alderman *et al.*, 2011; Campos *et al.*, 2013). This could be attributed to dilution effect of higher yield with added N.

Urea increased lignin concentration for September 2011 samples, with lignin in the highest urea application rate (4.68% of DM) higher than plots not receiving urea (4.56% of DM). Urea effect on lignin may be a product of maturation, because N increases relative maturity due to faster growth (larger plants) (Van Soest, 1996). This is also reflected in lower water content in September 2011 samples (longer harvest interval) relative to December 2012 samples (Table 1). This urea effect on lignin is unlikely to be consistent from harvest-to-harvest, especially with shorter cutting intervals in which relative maturity levels are less divergent as demonstrated by the lack of a treatment effect in December 2012.

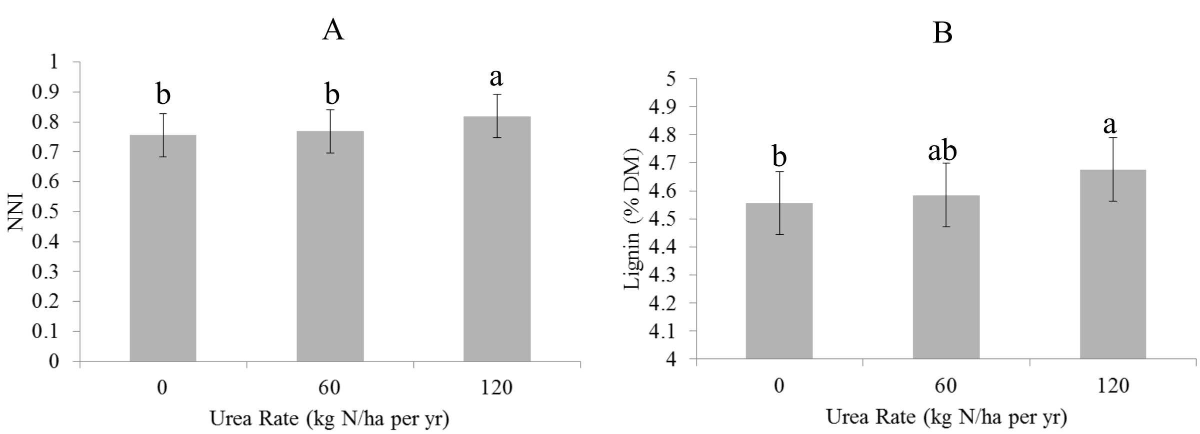

Figure 2 Least square means for effect of urea on nitrogen nutrition index (NNI, A, P=0.0065) and lignin (B, P=0.0469) in Mulato II forage samples collected in September 2011 and December 2012 (n=180). Error bars are 1 SE of the mean. Bars connected by the same letter in each bar graph are not significantly different.

Variation in DM concentration could be large from season-to-season (i.e., forage DM is higher during dry periods and periods with longer harvest intervals). Temperature, rainfall, and harvest interval effects on DM concentration are stronger and more important than compost as demonstrated by their effects in the full DM model from all harvests (n=24). Fixed effects included compost rate (P=0.0975), cumulative rainfall in each growth period (P<0.0001), harvest interval (P<0.0001), mean daily temperature in harvest interval (P<0.0001), and the number of plants in each plot (P=0.0108). Dry matter concentration decreased with compost applied from 22.1% when no compost was added to 21.3% when 24 Mg DM/ha per yr of compost was applied. Dry matter concentration increased by 0.332% for every 1°C increase in mean temperature and by 0.152% for each additional day between harvests, and decreased by 0.191% for each additional plant in the plot and by 0.00526% for each 1-mm increase in rainfall. Block (farm) explained 31% of residual variance while plot explained less than 1%, suggesting relatively large farm differences and consistent response to main effects.

3.2 Nutrient yield

Dry matter yield in September 2011 was nearly three times higher than in December 2012. Nutrient yield for measured nutritive value parameters increased with urea application, and was primarily a function of DM yield response to urea (Table 3). Compost application did not impact nutrient yield and a compost x urea interaction was not detected. Nutrient yield was higher for the highest urea application rate than for plots receiving the intermediate urea rate and those not receiving urea. The only exception was K yield, which was higher for the highest urea application rate than without urea. Potassium yield for plots receiving urea at 60 kg N/ha per yr did not differ from other urea application rates.

Nutrient yield from Mulato II could be sufficient to support supplementation of smallholder cattle production with forage produced in backyard plots (Table 3). Precise ration balancing combining Mulato II supplementation with other available diet components will be necessary to ensure adequate nutrient supply for cattle production in these systems.

Table 3 Geometric means (back-transformed least square means from log- or square root-transformed responses) for Mulato II nutrient yield as impacted by urea (n=180). Models include urea main effect, harvest period covariate, and block as a random effect.

Nutrient Yield	units	P	Urea Application Rate[a] kg N/ha per yr		
			0	60	120
Crude protein	Mg/ha per yr	0.0003	2.14b	2.26b	2.65a
Acid detergent fiber	Mg/ha per yr	0.0009	4.28b	4.69b	5.58a
Neutral detergent fiber	Mg/ha per yr	0.0008	7.70b	8.42b	10.0a
Digestible dry matter	Mg/ha per yr	0.0002	7.61b	8.32b	9.88a
Sugar	Mg/ha per yr	0.0005	0.831b	0.911b	1.08a
Ash	Mg/ha per yr	0.0004	1.38b	1.53b	1.80a
Non-fiber carbohydrates	Mg/ha per yr	0.0006	1.03b	1.13b	1.34a
Lignin	kg/ha per yr	0.0003	533b	585b	709a
Fat	kg/ha per yr	0.0005	434b	470b	558a
Ca	kg/ha per yr	0.0002	98.3b	104b	124a
P	kg/ha per yr	0.0003	46.4b	50.4b	59.7a
Mg	kg/ha per yr	0.0001	49.7b	52.9b	63.0a
K	kg/ha per yr	0.0058	255b	283ab	327a
S	kg/ha per yr	<0.0001	31.9b	34.6b	42.3a
Total fatty acids	kg/ha per yr	0.0005	231b	248b	298a

[a] Means connected by the same letter in each row are not significantly different.

Harvest intervals for the periods analyzed for nutritive value in this experiment (49 d and 32.5 d) produced high nutritive value forage for supplementary cattle consumption. A favorable approach to harvest high nutritive value forages may be the application of short cutting intervals to harvest at an earlier developmental stages (stem elongation or boot stages), although intervals less than three weeks negatively impact persistence and should be avoided (Vendramini *et al.*, 2014). Future field research should target the harvest interval component in the study region. The impact of fertilization on most nutritive value parameters typically used in ration formulation from this experiment is not sufficient to support harvest management decisions.

With limited resources in smallholder systems, our results suggest that it will be more feasible to manage forages for high yield with compost and urea fertilization than for high nutritive value. Further supporting this conclusion, a review of pastureland studies demonstrated that 60 to 90% of variation in average daily gain is due to forage quantity in pastures with high variation in forage mass (Sollenberger and Vanzant, 2011) such as those in rain-fed forage systems of south-central Vietnam.

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APPENDIX 1: Main effect P values for nutritive value

Table A1 Main effect P values for Mulato II forage nutritive value responses in mixed models. Separate models were generated for September 2011 and December 2012 samples (n=90 for each period). Models contain fixed effects of compost, urea, and compost x urea interaction, and block as a random effect. Significant effects are highlighted in gray.

Parameter	September 2011			December 2012		
	Compost	Urea	Compost x urea	Compost	Urea	Compost x urea
Dry matter (%)	0.0095	0.2663	0.8596	0.3898	0.9211	0.9910
Crude protein (% DM)	0.3834	0.8025	0.9676	0.7010	0.7360	0.5471
Nitrogen nutrition index	0.2106	0.0051	0.6658	0.7029	0.2277	0.1867
Acid detergent fiber (% DM)	0.3448	0.7660	0.8115	0.3997	0.9820	0.6742
Neutral detergent fiber (% DM)	0.5459	0.6356	0.8863	0.8959	0.5463	0.6675
Lignin (% DM)	0.6133	0.0469	0.6304	0.4513	0.3931	0.3557
Neutral detergent fiber digestibility at 30 h (% NDF)	0.3528	0.2138	0.8201	0.7776	0.9890	0.2768
In vitro dry matter digestibility at 30 h (% DM)	0.3928	0.3605	0.8902	0.9111	0.8955	0.3339
Acid detergent insoluble crude protein (% CP)	0.8989	0.7167	0.9327	0.8890	0.4569	0.2778
Neutral detergent insoluble crude protein (% CP)	0.7459	0.8413	0.8198	0.1993	0.7265	0.4905
Soluble protein (% CP)	0.4591	0.9244	0.9483	0.5918	0.8618	0.3208
Fat (% DM)	0.2136	0.2644	0.8717	0.7820	0.9574	0.5011
Ash (% DM)	0.2562	0.3903	0.4520	0.0385	0.4924	0.5476
Ca (% DM)	0.3692	0.6371	0.7231	0.3269	0.1422	0.0918
P (% DM)	0.0583	0.8089	0.9528	0.2210	0.6370	0.6447
Mg (% DM)	0.1355	0.1410	0.6443	0.0010	0.1734	0.5611
K (% DM)	0.0231	0.8744	0.3860	0.0831	0.1881	0.5453
S (% DM)	0.1431	0.3138	0.7281	0.4430	0.2771	0.7126
Sugar (% DM)	0.0577	0.3467	0.4328	0.4104	0.5762	0.5334
Non-fiber carbohydrates (% DM)	0.1540	0.1919	0.7959	0.0497	0.5853	0.8705
Digestible dry matter (% DM)	0.3453	0.7650	0.8117	0.4005	0.9818	0.6708
Kd rate (Digestion rate) (%/h)	0.5592	0.6437	0.9715	0.9682	0.7522	0.0966
Total fatty acids (% DM)	0.5695	0.6840	0.7654	0.7751	0.3750	0.5782

APPENDIX 2: Treatment interaction means for nutritive value parameters

Table A2 Mulato II forage nutritive value parameter means for compost x urea interaction.

	Compost Treatment (Mg DM/ha per yr) / Urea Treatment (kg N/ha per yr)														
Parameter	**0**	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**	**0**	**4**	**8**	**12**	**24**
	0	**0**	**0**	**0**	**0**	**60**	**60**	**60**	**60**	**60**	**120**	**120**	**120**	**120**	**120**
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Dry matter (%)	21.0	20.4	20.8	19.8	19.6	20.9	20.5	20.5	20.5	19.7	20.5	20.3	20.4	19.8	19.6
Crude protein (% DM)	15.5	15.1	14.7	14.9	15.1	14.8	15.5	14.7	14.6	15.3	14.7	15.3	15.0	15.0	15.8
Nitrogen nutrition index[a]	0.771	0.757	0.718	0.762	0.769	0.736	0.822	0.747	0.790	0.748	0.776	0.799	0.800	0.819	0.902
Acid detergent fiber (% DM)	34.3	34.7	35.0	35.0	35.1	34.8	34.4	35.3	35.0	34.8	34.9	34.6	35.0	35.2	34.9
Neutral detergent fiber (% DM)	62.0	62.5	62.6	63.1	62.6	62.4	62.1	62.9	62.8	62.3	63.0	62.2	62.8	62.5	62.2
Lignin (% DM)	4.33	4.35	4.38	4.33	4.35	4.26	4.35	4.45	4.42	4.34	4.39	4.36	4.45	4.51	4.52
Neutral detergent fiber digestibility at 30 h (% NDF)	61.5	61.3	61.6	61.0	61.4	61.5	61.9	61.3	61.1	61.0	61.1	61.3	60.6	60.9	61.6
In vitro dry matter digestibility at 30 h (% DM)	76.1	75.8	75.9	75.3	75.8	75.9	76.3	75.6	75.5	75.6	75.5	75.9	75.2	75.5	76.1
Acid detergent insoluble crude protein (% CP)	0.763	0.738	0.746	0.728	0.752	0.735	0.757	0.757	0.756	0.751	0.757	0.755	0.742	0.748	0.779
Neutral detergent insoluble crude protein (% CP)	3.41	3.35	3.28	3.23	3.34	3.32	3.44	3.32	3.26	3.28	3.22	3.40	3.34	3.31	3.50
Soluble protein (% CP)	31.6	32.1	31.0	30.7	31.5	31.0	30.6	31.5	30.6	33.1	30.6	32.6	31.2	31.2	32.6
Fat (% DM)	3.54	3.52	3.51	3.57	3.52	3.47	3.57	3.45	3.45	3.53	3.48	3.52	3.53	3.44	3.50
Ash (% DM)	11.0	11.1	11.3	11.2	11.7	11.5	11.2	11.5	11.1	11.6	11.1	11.4	11.3	11.2	11.3
Ca (% DM)	0.704	0.663	0.690	0.665	0.683	0.668	0.714	0.696	0.660	0.664	0.690	0.732	0.662	0.698	0.705
P (% DM)	0.373	0.383	0.377	0.373	0.384	0.373	0.378	0.377	0.367	0.384	0.362	0.374	0.376	0.372	0.386
Mg (% DM)	0.347	0.348	0.348	0.345	0.349	0.338	0.357	0.353	0.339	0.343	0.351	0.368	0.345	0.354	0.367
K (% DM)	1.90	2.08	2.11	2.22	2.25	2.21	2.05	2.14	2.09	2.27	2.05	1.95	2.07	2.19	2.19
S (% DM)	0.264	0.264	0.253	0.263	0.263	0.256	0.265	0.257	0.252	0.265	0.264	0.272	0.261	0.261	0.273
Sugar (% DM)	7.29	7.22	7.23	7.15	6.89	7.09	7.25	7.04	7.21	7.10	7.19	7.34	7.00	7.10	7.02
Non-fiber carbohydrates (% DM	9.31	9.05	9.26	8.67	8.50	9.17	9.00	8.82	9.40	8.69	8.97	8.86	8.82	9.12	8.49
Digestible dry matter (% DM)	62.2	61.9	61.6	61.6	61.5	61.8	62.1	61.4	61.6	61.8	61.7	62.0	61.6	61.5	61.7
Kd rate (Digestion rate) (%/h)	4.09	4.05	4.12	3.98	4.07	4.07	4.14	4.08	4.06	4.02	4.03	4.06	4.00	4.08	4.17
Total fatty acids (% DM)	1.96	1.88	1.88	1.89	1.91	1.85	1.97	1.82	1.80	1.95	1.86	1.94	1.86	1.89	1.95

CHAPTER 3: CHARACTERIZATION OF CATTLE MANURE VALUE CHAINS IN SOUTH-CENTRAL VIETNAM

Abstract

Cattle manure value chains play an important role in smallholder crop-livestock systems in south-central Vietnam. Lowland cattle farmers sell manure through a network of chain participants including small-scale collectors, lowland traders, and highland traders, to pepper, coffee, dragon fruit, and rubber farms in the Central Highlands and southeast coast. This study describes cattle manure value chains originating in two representative lowland communes, Nhơn Khánh (NK) and An Chấn (AC). Semi-structured interviews with value-chain participants between April and September 2013 collected information from lowland farmers (n=101), manure collectors and traders (n=27), rubber companies (n=2), highland farmers (n=50), and dragon fruit farmers (n=20) about cattle management, manure-related labor, manure transactions, and fertilizer and manure use. Lowland cattle owners were selected using stratified random sampling. Farmer interviews identified subsequent value chain participants. Most farmers interviewed reported manure sales (AC=76%, NK=90%), and reported sales of 62% of available farm manure with SE ± 2.9%, with the remainder being composted and used for crop fertilization. Farmers dried manure for approximately 4 days to prepare it for sale. Dry manure was bagged and sold to manure collectors and traders who arranged transfer to highland farmers, southeast coastal farmers, or other traders. Manure was sold by the bag (standard bag volume in AC = 41.7 L, NK = 71.4 L) in the lowlands and mostly by the cubic meter in destinations. Sale price in farmer-reported transactions was \$16.27 with SE ± \$0.22/m^3. Farmers reported most manure sales between February and August (during NK and AC dry season) when demand exists for organic amendments in the highlands. From NK, approximately 80% of manure sold was transported to Gia Lai Province and 20% to Đắk Lắk Province in the Central Highlands. From AC, 70% was transported to Gia Lai, 10% to Đắk Lắk, 5% to Đắk Nông. Fifteen percent was

transported to dragon fruit farmers in Bình Thuận Province late in the year. Prior to application, highland farmers purchasing manure often prepared a 45-day compost with manure and other amendments including potassium, urea, coffee pulp, and a commercial probiotic. Value chain participant net incomes ranged from over \$13,000/yr for traders to near \$100/yr for lowland farmers, and returns to labor were just over \$2/h for traders and \$0.50/h for lowland farmers. Manure trade is an important source of supplementary income for farmers and a primary livelihood activity for traders. This study generated descriptive information about manure value chains in Vietnam that can inform analysis of value chain dynamics via simulation modeling.

1. Introduction

Large ruminants are key assets for smallholder farmers in developing countries. Cattle contribute to poverty reduction as investments and buffers during times of economic uncertainty (Owen, 2005). Additional benefits include animal traction, fuel and fertilizer from manure, and income from milk, meat and hides. The use of cattle manure as organic fertilizer and fuel (heating, cooking, and biogas production) is common in developing countries. Informal manure trade is a less common, but very important component of smallholder crop-livestock systems in some regions (Hoffmann *et al.*, 2014). Manure sales provide a supplementary source of cattle byproduct income for participating farmers, the importance of which for income and nutrient balance is not well documented in most regions with active manure trade.

Recent large ruminant manure trade systems have been reported for India (Köhler-Rollefson, 2005; Harsdorff, 2012), Kenya (Lekasi *et al.*, 2001; Kirigia *et al.*, 2013), Nigeria (Olayide *et al.*, 2009), and Indonesia (FAO, 2005), although few studies have explored manure value chains in depth. Harsdorff (2012) assessed the economics of cow manure in detail, and reported that the informal industry is important to the national economy in India, although manure is commercially underutilized (i.e., about half of potentially available manure is unused, washed away or applied to fields) rather than being processed for commercial use. In some areas of India, one manure-related job exists for every two jobs in the milk industry and manure makes up about 10% of the total economic value from milk production systems, a proportion that could rise with higher commercially productive use of manure (Harsdorff, 2012). Nationally, Harsdorff (2012) estimated that an additional 2 million jobs could potentially be created by shifting informal manure cake production to commercial biogas production and directing underutilized manure for commercial use, for a total of 3.5 million jobs in dung industries (e.g., manure collection, commercial biogas production, electricity, fertilizer production). Lekasi *et al.* (2001) undertook a household survey on manure management in the Kenyan highlands, and reported the high value placed on manure by farmers (approximately 30% of annual milk production value), reflecting perceived soil fertility benefits. Kirigia *et al.* (2013) characterized cattle and small ruminant manure markets in Kenya by interviewing 60 pastoral households in four locations. This study demonstrated that most households participated in manure trade, and manure-related labor was undertaken primarily by women. They estimated manure value throughout the supply chain and observed seasonal variation in demand. Sales were either to traders, farmers, or larger horticultural farms, which often occurred via traders. These previous studies have focused on the potential economic benefits of manure trade and the overall structure of local trade systems, but have not targeted a detailed description of manure participants, manure use, regional manure flows, manure-related labor, or the economics of manure trade for different actors.

In Vietnam, cattle produced on smallholder farms in the south-central coast provide income from live animal sales and contribute to national beef supply (Ba *et al.*, 2013; Parsons *et al.*, 2013). Cattle also provide animal traction and produce manure for crop fertilization and sale. Cattle manure is an important on-farm organic resource, and manure management poses a significant decision. On the south-central coast, cattle manure can be applied to cash crops (e.g., rice, peanuts, and cassava), cultivated forages, or sold. Cattle manure value chains may play an important role in smallholder crop-livestock systems in Vietnam, although existing manure value chains have not yet been described or evaluated in detail (Birch *et al.*, 2014). Manure supply chains have been observed in several studies, including a manure management survey that indicated hog manure trade (Vu *et al.*, 2007), and assessment of economic potential for further development of a hog manure commodity chain in north Vietnam (Colson and Boutonnet, 2006). In the Central Highlands (Đắk Lắk Province), a participatory appraisal of rural livelihoods indicated cattle manure sales between cattle owners and coffee farmers (Cramb *et al.*, 2004). Dan *et al.* (2004) discovered an unregulated manure market in southeast Vietnam (near Ho Chi Minh City) during an assessment of options for recycling of animal waste. Manure trade provided a favorable option for recycling where markets existed and transportation issues could be overcome.

Manure is minimally treated in the overall value chain literature, despite its importance in smallholder crop-livestock systems of Asia, Africa, and Latin America (Hoffmann *et al.*, 2014). Thus, we aim to improve knowledge of manure value chains by describing chains originating in two lowland communes of south-central coastal Vietnam, one in Bình Định Province and another in Phú Yên Province. We characterize manure value chain participants, locations, roles and manure use by applying a traditional survey-based approach to value chain description and quantification (Kaplinsky and Morris, 2001; Rich *et al.*, 2011). In this first stage assessment, we target the linkages and structure of the value chain with quantification of net incomes, expenditures, and manure use. Detailed results for participants in the value chain network shed light on the economics of manure transactions, overall financial implications, and labor. Researchers and development agencies could benefit from this information by better understanding the role of cattle manure as an important byproduct in smallholder crop-livestock and socioeconomic systems and the impact of manure value chain participation on different actors. It could also provide insight about nutrient redistribution via manure trade at the landscape scale and implications of manure trade for sustainable nutrient management in smallholder crop-livestock systems.

2. Methods

2.1 Survey methodology

Two lowland communes with active manure trade in south-central coastal Vietnam were selected as manure value chain initiation points based on recommendations from researchers at Huế University of Agriculture and Forestry: Nhơn Khánh Commune (NK) in Bình Định Province and An Chấn Commune (AC) in Phú Yên Province. Informal discussions with agricultural extension educators and village leaders generated preliminary value chain maps and identified key actors and manure traders in lowland communes. Agricultural extension educators serving each commune assisted with identification of the lowland farmer sampling frame, a list of all households owning cattle and number of animals owned. Overall household cattle ownership in AC (n=390 households own cattle) was 3.7 animals with SD ± 2.8 animals and 2.2 ± 1.1 animals in NK (n=1045 households own cattle). Lowland cattle owners were selected using a stratified random sampling approach, with strata defined by population proportion in each household herd size group (3 strata: 1 to 3 head, 4 to 6 head, and > 7 head). Stratification ensured representation of the large herd size group in selected households.

Data collection was undertaken between April and September 2013 using semi-structured in-person interviews (Appendix 2) with value chain participants (Table 1). Participants included manure suppliers (lowland cattle owners), end users (highland coffee and pepper farmers, rubber companies, and southeast coastal dragon fruit farmers), and manure traders. Manure collectors and traders were divided into four categories. (1) *Lowland compost manure traders* who purchase and resell composted manure locally; An Chấn compost collectors were oxcart owners and drivers, and undertook compost manure trade as part of their local transport business. (2)

Lowland small-scale collectors and oxcart transporters who purchase bagged manure from surrounding farms as a service to larger lowland traders and store it at a collection point for pickup by larger traders; (3) *Lowland traders* who purchase manure from farmers and small-scale collectors, store it, and resell it to distant locations; and (4) *Highland traders* who purchase manure from lowland and highland locations to resell in the highlands.

Table 1 Manure value chain participants interviewed in the study.

Participant type	Locations	n
Lowland cattle owners	AC and NK[a]	101
Lowland compost manure traders	AC[a]	2
Small-scale lowland collectors	AC and NK[a]	13
Lowland traders	AC and NK[a]	8
Highland traders	Gia Lai Province	4
Highland pepper and coffee farmers	Đắk Lắk and Gia Lai Provinces	50
Dragon fruit farmers	Bình Thuận Province	20
Rubber companies	Gia Lai and Bình Thuận Provinces	2

[a] AC = An Chấn Commune in Phú Yên Province; NK = Nhơn Khánh commune in Bình Định Province.

Responses to survey questions represent farmer and trader perceptions, which (as in all recall surveys) may differ from measured quantities. A street-clothed member of the regional police force accompanied researchers during collector and trader interviews in NK. Interviews were conducted in Vietnamese, and duration ranged from 10 min to 90 min, with average duration of approximately 40 min. Questionnaires consisted of modules including: (1) cattle management (lowland farmers only), (2) manure-related labor (lowland farmers and traders only), (3) manure transactions, (4) annual manure trade, and (5) fertilizer and manure use (lowland and destination farmers). Transaction data may be more accurate than annual data for most value chain actors, because transactions represented specific recent buyer – seller interactions, whereas overall sales and purchases were estimated on an annual basis from approximate cumulative monthly and seasonal purchases and sales.

It was not possible to obtain representative samples for value chain participants downstream from product origin (lowland farms), because information about downstream participants was unavailable prior to upstream interviews, and a bounded sampling frame did not exist for downstream participants. Thus, our downstream sampling processes aimed to obtain representative samples for predominant actors, including all lowland manure collectors and traders operating in NK and AC communes, highland traders and rubber companies identified by lowland farmers and collectors, and predominant destinations identified by collectors, traders, and lowland farmers. Furthermore, end users procured manure from other sources than AC and NK communes. Thus, we believe that although the selection process was not representative for some downstream participants (e.g., end users), it enabled effective downstream chain characterization for districts in primary value chain destinations.

Beginning with lowland cattle farmers in NK (n=51) and AC (n=50), subsequent value chain participants were identified during preceding interviews. All lowland traders and collectors identified were interviewed. Higher-capacity lowland traders identified predominant manure destinations, including highland traders and specific destination districts and communes. The five most frequently mentioned highland destinations and two secondary destinations in Bình Thuận were selected for end user sampling. Ten representative households in villages in a single commune per district were then selected with the assistance of commune extension educators serving each destination commune. Four highland traders were also interviewed, three in person and one by phone.

Lowland farmer labor and returns to labor were evaluated based on reported hours invested in steps to prepare and sell one cubic meter of manure, and the revenue generated from manure sales for each hour invested in preparation and sale. Labor invested per cubic meter of manure sold was calculated by dividing total estimated hours for yearly manure-related labor for sold (dry) manure by total annual manure revenue for each household interviewed. Similarly, returns to labor ($/hr) were calculated as total annual manure revenue divided by total estimated hours for yearly manure-related labor. A limited number of very high estimates inflated means. Thus, medians were calculated to more accurately reflect labor and returns to labor.

Annual revenues for traders and collectors were estimated as the difference between the product of reported annual manure sale prices and volume, and the product of purchase price and volume. Reported hired labor costs and transport costs were then subtracted from revenue to estimate net incomes from manure trade activities. Returns to labor for traders were calculated as net income divided by annual manure trade labor.

Annual manure volumes sold from lowland communes were estimated in two ways: (1) annual household sales volumes reported by lowland farmers were multiplied by number of households selling manure, and (2) cattle numbers and factors influencing manure available for sale in lowland communes according to: *Commune cattle population x 700 kg manure DM/cow per yr x fraction households selling manure x ((1 – daily grazing hours/24) x fraction households using grazing management + (1 – fraction households using grazing management)) x fraction available manure sold x kg dry manure per* m^3. Estimates based on overall cattle numbers were more conservative and were used to estimate aggregated manure flows through the value chain.

2.2 Manure sample collection and chemical analysis

Manure samples of sale product were collected from AC and NK farmers. Samples were dried at 60°C in a forced-air oven to determine dry matter concentration. Chemical composition was tested at the Soil Science Laboratory at Huế University of Agriculture and Forestry. Tests included organic matter (Walkley-Black method, as described in Nelson and Sommers (1996)), total N (Kjeldahl methods, as described in Bremner (1996)), and total P (samples were ashed at

450°C and then digested with perchloric acid and nitric acid, as described in Kuo (1996)). Price of nutrients in manure and fertilizer was calculated as *product price per kg / kg nutrient per kg product DM*.

2.3 Statistical analysis

Statistical analyses were executed in JMP® Pro 11.2.0 (SAS Institute Inc., 2013) to generate descriptive statistics and bivariate and multivariate statistical models (ANOVA) to evaluate differences among locations, value chain actors and other factors impacting economics of manure trade. Fixed effects were tested individually in mixed lowland farmer transaction models with household as a random effect to account for non-independence of transactions within household. Random effects were estimated using restricted maximum likelihood and variance components. The sampling unit was the household for farmers and the interviewee for traders and collectors. Mean differences ($P \leq 0.05$) were assessed with Tukey's adjustment for multiple comparisons or a t-test for comparison of two groups. Retrospective power of tests for mean differences was calculated for fixed effects models. Residuals were evaluated for normality, and log and square root transformations were applied to response variables as needed to improve model fit. Geometric means and 95% confidence intervals were calculated for transformed data in lieu of means and standard errors.

3. Results

3.1 Manure flows

Lowland cattle farmers often choose to sell cattle manure during the dry season. Via a network of chain participants, manure product reaches the Central Highlands and southeastern coastal regions where it is used as an organic matter soil amendment for black pepper, coffee, dragon fruit, and rubber production by farmers and companies. Estimates of manure flows and destinations were determined from interviews with lowland traders and small-scale lowland collectors in AC and NK communes (Figure 1). From NK, approximately 80% of manure sold was transported to Gia Lai province and 20% to Đắk Lắk Province in the Central Highlands. From AC, 70% was transferred to Gia Lai, 10% to Đắk Lắk, and 5% to Đắk Nông in the Central Highlands. Fifteen percent of AC manure was used in dragon fruit farms in Bình Thuận Province late in the year (August onward). Highland destinations included pepper farms, coffee farms, and rubber companies.

The sampling process identified manure flows and associated value chain participants and steps between communes in the south-central lowland coast and highland and southeast coastal destinations (Figure 2). Detailed results for participants in the value chain network shed light on the economics of manure transactions, overall financial implications, and labor.

3.2 Value chain participants

3.2.1 Lowland farmers

Smallholder cattle farmers interviewed in lowland communes owned less than four head on average (AC = 3.9 head with SE ± 0.38 head, NK = 2.7 head ± 0.22 head) and owned and rented 0.268 ha with SD ± 0.195 ha of land. Most farmers reported at least one manure sale (AC=76%, NK=90%), and those that sold manure reported sales of 62% with SE ± 2.9% of available farm manure. Proportion of available manure sold did not depend on commune (P=0.9611); however, most owners (62%) took cattle out to graze on communal land or vacant fields during the day and few farmers collected manure during grazing periods, so some manure production was not available for sale and crop application. Daily grazing time in AC was 7.5 h with SE ± 0.28 h, a distance of 1.6 km with SE ± 0.13 km from the household. The daily grazing time in NK was 3.6 h with SE ± 0.26 h, a distance of 269 m with SE ± 31.5 m from the household. Only two AC farmers and three NK farmers collected manure when cattle were out grazing. The two AC farmers reported collection of manure from cattle owned by other farmers to augment manure available for sale. Daily grazing was supplemented near the household with ingredients consisting primarily of grass, rice straw, rice, and rice bran (Appendix 2 Table A3). All animals were crossbred in NK. Cattle in AC were a mix of crossbred and local yellow cattle.

Manure not sold was retained and composted for use as fertilizer for crop and forage production (Appendix 1 Table A1), and manure retention for agricultural use was the most important reason for not selling any manure in both communes (14% of farmers). Most farmers composted cattle manure during the late dry season (August) and the rainy season (September to December), and applied prepared compost on the first rice crop (winter-spring), second rice crop (summer-fall), or forage grass (Appendix 1 Table A1). Compost was most commonly applied to rice and forage grasses at rates below 10 Mg DM/ha per yr. The most commonly reported manure management challenge was lack of a manure pit to collect manure during the rainy season (11% of farmers). Manure loss during high rainfall periods due to flooding was problematic for these farmers.

In AC, the most commonly reported reasons for selling manure were to cover daily expenses (14% of AC farmers) and to increase household income (14%). Three farmers indicated that they sold due to manure excess after accounting for all agricultural needs. Farmers in NK most frequently reported excess manure after accounting for agricultural needs as the reason driving sales (29% of NK farmers). Increasing income (12% of NK farmers) and purchasing cattle feed (e.g., rice straw) (20% of NK farmers) with cash from manure sales were also important. Other reasons for manure sales mentioned in both communes were challenges in transporting manure to distant fields, labor shortfalls for manure collection and application, to purchase fertilizer, and to buy vegetables.

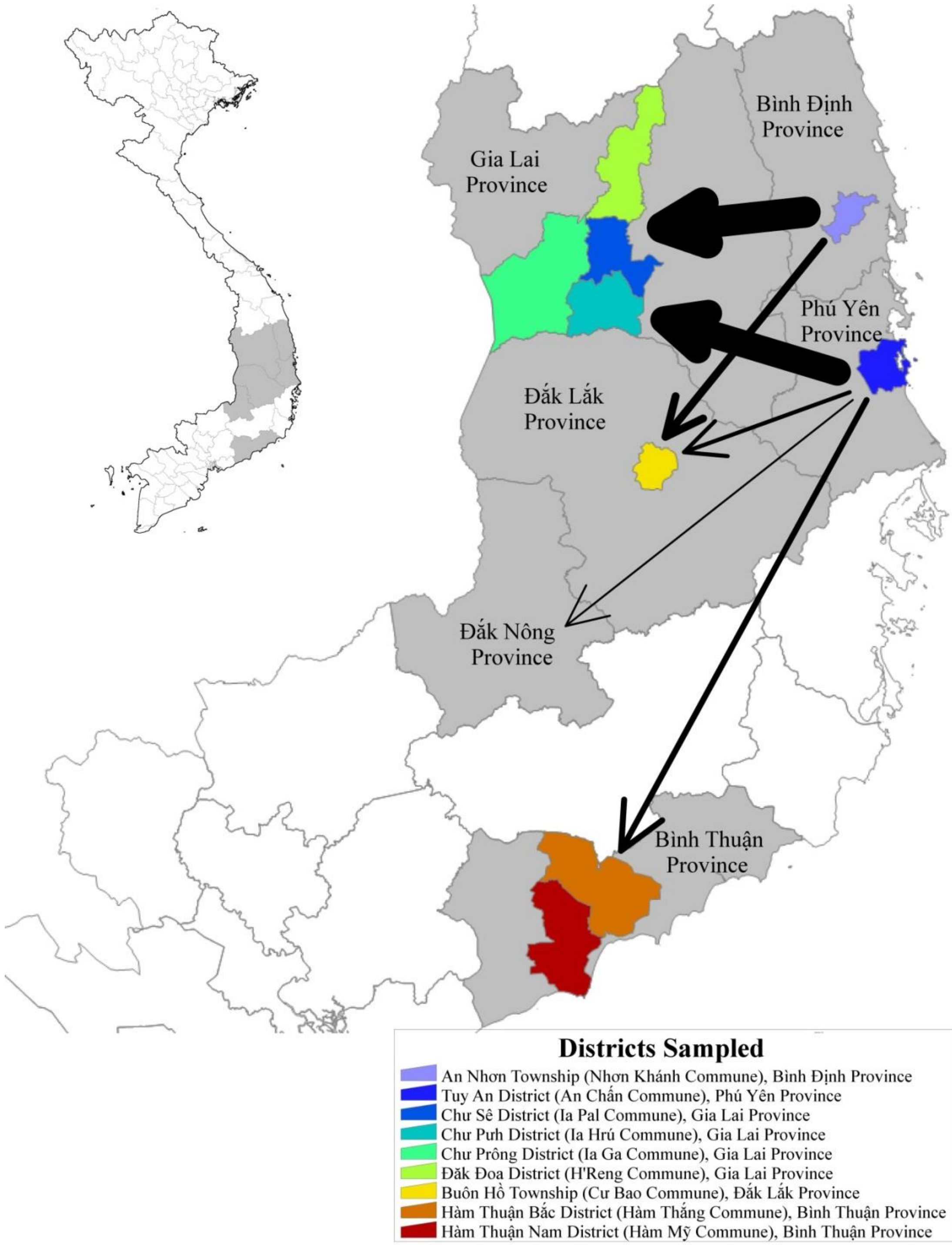

Figure 1 Map of study areas in Vietnam with whole country inset. Provinces are gray. Districts sampled are colored areas within provinces. Manure flow from sampled lowland communes to principal destinations is denoted by black arrows, and arrow width is proportional to flow size reported by lowland manure traders.

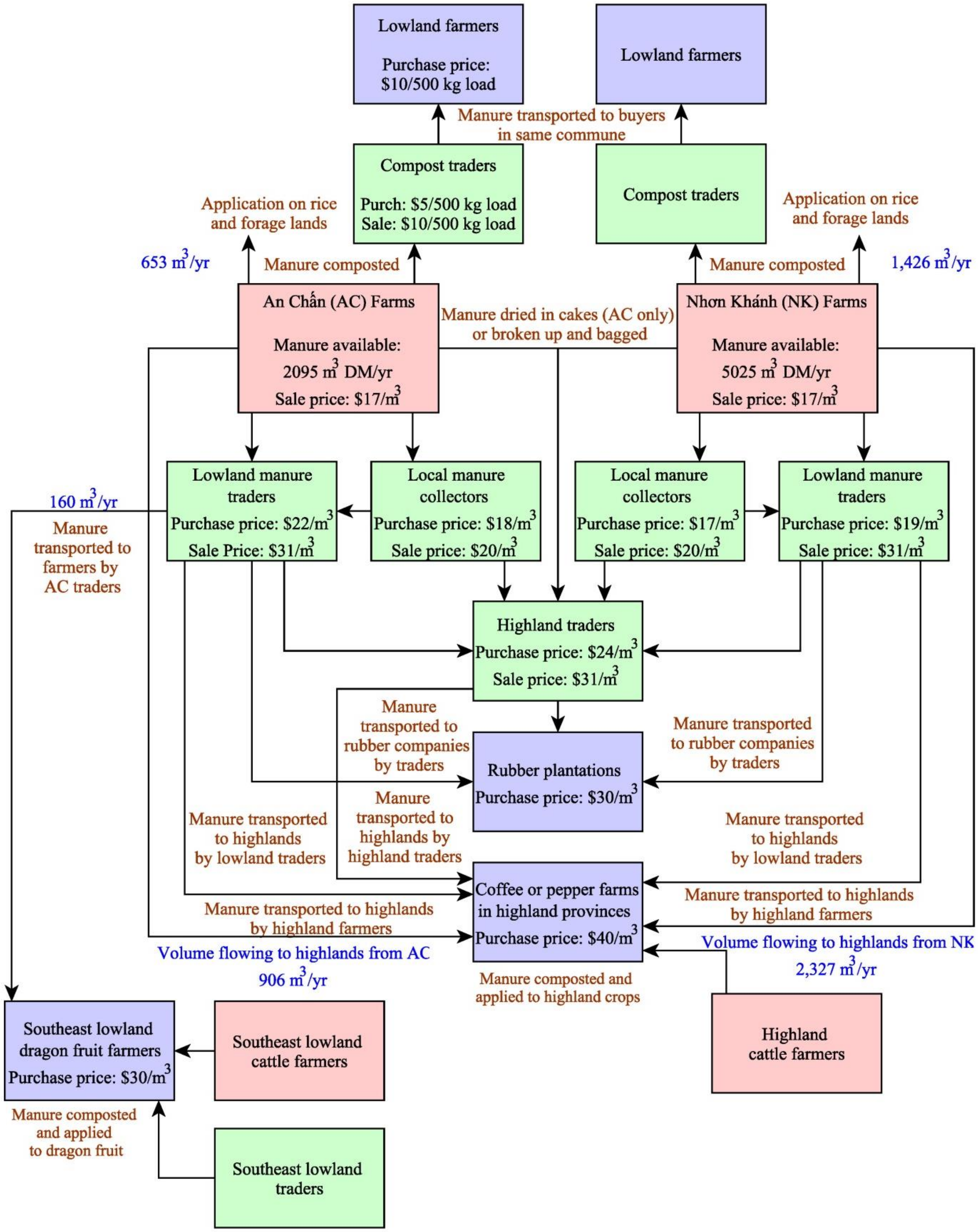

Figure 2 Probable manure flows (arrows), associated participants and prices (in boxes; pink = manure sources, green = traders, blue = end users), aggregated volumes (blue text), and steps (brown text) between lowland communes and highland (pepper and coffee farmers) or southeast coastal (dragon fruit farmers) destinations.

Farmers prepared manure for sale by drying it on the ground or in cakes on posts, branches, or walls for approximately four days under favorable (dry, sunny) conditions. Dry manure product (89% DM with SD ± 0.03% DM, 187 g OM/ kg DM with SD ± 56 g OM/kg DM, 12.7 g N/kg DM with SD ± 3.7 g N/kg DM, and 5.9 g P_2O_5/kg DM with SD ± 2.7 g P_2O_5/kg DM) was bagged and sold to local manure collectors who arranged transfer to traders or farmers in the highlands. Manure was sold by the bag, and average bag volumes were determined from local traders on the basis of bags per cubic meter (AC and NK traders reported 24 and 14 bags/m^3 respectively, for bag volume of 41.7 L in AC and 71.4 L in NK). Full bag weights were reported at approximately 15 kg/bag in AC and 25 kg/bag in NK. Empty bags were provided to farmers by lowland traders.

Farmers were asked if manure sold was different than manure retained for household use. Among 65 respondents that said they sell and use manure, 92% indicated that composted manure was retained for household use while dried manure (broken-up or cakes) was sold. Most farmers (54% of 65 question respondents) believed that the quality of dry (sold) manure was equal to manure retained for household use, while 46% believed composted manure was higher quality than dry (sold) manure.

Many farmers would like to continue to dry and sell manure during the rainy season (51% of 79 question respondents), but low prices, buyer scarcity, and difficulty drying manure inhibited sale. Farmers would use manure for crop production (75% of 76 question respondents) and/or store it until the following dry season (37% of question respondents) if they could not sell it. Some farmers (21% of all farmers) reported intentional stockpiling of manure during the rainy season to dry and sell early in the dry season. Two farmers shared manure with other local farmers during the rainy season or sold composted cattle manure during the rainy season. Two farmers traded cattle manure with other farmers for forage or rice straw to feed cattle. Thus, non-monetary manure trade was uncommon.

Farmers were asked about the impact of not selling manure on farm nutrient management. All question responses (n=61) included increased soil organic matter and soil nutrients in agricultural fields, fostering improved plant development and decreased reliance on chemical fertilizers.

3.2.1.1 Lowland farmer manure transactions

Sale price[1] in farmer-reported transactions (n=130 transactions reported by 82 farms) was \$16.27/m^3 with SE ± \$0.22/m^3. Mean price did not differ by commune (P=0.0623), manure type (cakes or broken-up) (P=0.5517) or buyer type (local trader or small-scale collector) (P=0.0662). All NK manure was spread to dry on the ground, roadside, or cement slabs. Approximately 50% of AC manure was formed into cakes while the rest was spread to dry on the ground or roadside. Drying times were approximately four days under favorable (sunny, dry) conditions. Manure price in AC cake transactions (n=39) was significantly lower (\$16.00/m^3 with SE ± \$0.36/m^3) than manure that was spread on the ground to dry (n=20) (\$18.23/m^3 with SE ± \$0.51/m^3) (P=0.0015). Household explained just 2% of residual variance in this model, suggesting consistent transaction prices within individual households. Only one farmer (AC) said that selling a minimum of 15 bags (0.625 m^3) per transaction would save time.

Manure prices reported in transactions indicated highest prices early in the year (Figure 3), with prices declining quadratically (P=0.0001) from April onward. Most reported transactions (71%)

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[1] Monetary values reported during interviews were in Vietnam đồng (VND). An approximate exchange rate in 2013 was 21,000 VND/USD, and value data are reported throughout the manuscript in USD using this conversion factor.

occurred in May and June, and variances were larger during these months than others reported, potentially due to high trader and collector competition during these months.

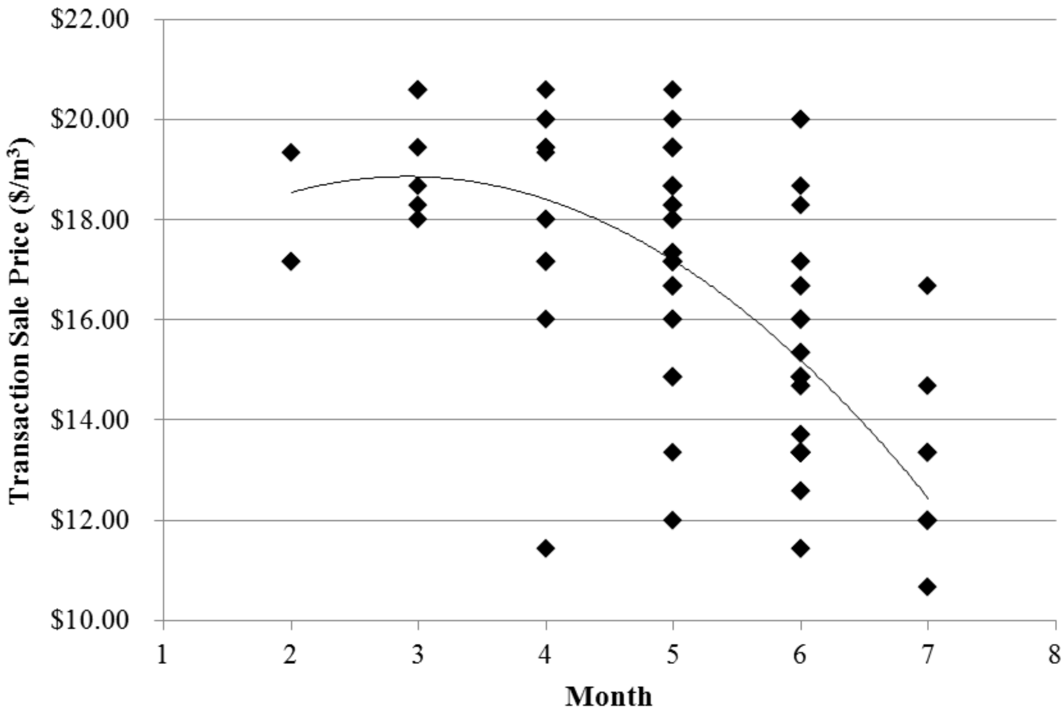

Figure 3 Transaction sale prices (n=130) reported by lowland farmers declined from April onward. The quadratic relationship is described by: $y = 0.384x^2 + 2.24x + 15.6$. Coefficient of determination (R^2) was 0.46.

Average transaction volume (n=132 transactions) was 0.724 m^3 with SE ± 0.0639 m^3, and revenue per transaction (n=130 transactions) was $12.26 with SE ± $1.17. Volume and revenue models included a continuous month covariate and household as a random effect. Household accounted for 65% and 69% of residual variance in volume and revenue models, respectively, indicating considerable transaction variation within households. Transaction volume and revenue differed by commune (P=0.0002, P=0.0018) and buyer type (P=0.0002, P=0.0002), with higher volumes and revenues for NK transactions and transactions with local traders, respectively. Commune and buyer type remained significant in multivariate mixed models containing a transaction month covariate with square root-transformed volume and revenue responses (Table 2), suggesting that these factors impacted volume and revenue when controlling for transaction time.

Table 2 Back-transformed least squares means (geometric means) and 95% CIs from square root-transformed lowland farmer manure transaction volume model (n=129) and square root-transformed transaction revenue model (n=128).

Effect	Category	Volume (m^3/transaction)			Transaction revenue ($/transaction)		
		Mean[a]	Lower 95% CI	Upper 95% CI	Mean[a]	Lower 95% CI	Upper 95% CI
Commune							
P<0.0001	NK	0.901 a	0.744	1.07	14.9 a	12.2	17.9
	AC	0.337 b	0.231	0.462	5.58 b	3.73	7.81
Buyer type							
P<0.0001	Local trader	0.812 a	0.639	1.01	13.7 a	10.7	17.2
	Small-scale collector	0.395 b	0.301	0.501	6.35 b	4.73	8.22

[a] Means not connected by the same letter for each effect in each model are significantly different.

One AC farmer purchased composted manure from a neighboring farm in May for $9.52/500 kg oxcart load (approximately 250 kg DM), plus a $2.38 delivery fee, for use in crop production. A second compost sales transaction was reported in May in AC. Four 500 kg oxcart loads (approximately 250 kg DM/load) were sold to a neighboring watermelon farmer for $8.57/load, including transportation.

3.2.1.2 Annual lowland farmer manure sales

Manure sales and prices were also reported on an estimated annual basis. The AC farmers sold 6.57 m^3/yr with SD ± 5.67 m^3/yr at an average price of $17.40/$m^3$ ± $1.31/$m^3$, yielding total annual household revenue of $116.36 ± $102.18. Farmers in NK sold 7.03 m^3/yr with SD ± 5.56 m^3/yr at an average price of $17.02/$m^3$ ± $1.04/$m^3$, yielding total annual household revenue of

\$119.98 ± \$93.94. Annual sales volumes, prices and revenues did not differ by commune. Annual revenue from manure sales was positively correlated with household cattle ownership (r=0.68) and owned and rented land (r=0.38). In a multivariate statistical model estimating square root-transformed annual manure revenue for lowland farms, significant effects included commune (P=0.0011), number of cattle owned (P<0.0001) and amount of land owned and rented (P=0.0251). In this model, annual revenue from manure sales in NK (\$125.04, 95% CI (\$107.36, \$144.07) was significantly higher than AC revenue (\$80.30, 95% CI (\$64.20, \$98.20)) (P=0.0251, Power=0.9172). Yearly commune sales volumes (AC = 1,947 m^3/yr; NK = 6,612 m^3/yr) may be overestimated by farmer-reported annual volumes, based on a comparison with probable volumes based on cattle numbers, farmers selling manure, grazing time, and proportion of available manure sold in each commune (AC = 1,066 m^3/yr; NK = 2,327 m^3/yr).

3.2.1.3 Lowland farmer labor and returns to labor

Manure preparation steps varied according to manure type (compost, broken-up, cakes). Composting of manure generally included daily transfer from the cattle shed to the manure pit or pile. Labor associated with turning the compost pile was considered minor and was not reported by farmers. Crop application of composted manure was not included.

Labor to prepare and sell broken-up manure included: (1) daily transfer of fresh manure from cattle shed to manure pile, (2) spreading of fresh manure on a cement slab, on the ground, or roadside to dry in a broken-up form, (3) a minimum of four days of sun drying (duration is weather dependent), (4) bagging in large sacks, and (5) sale. Sale-related labor was included only if farmers delivered bags to buyers. Many local collectors and traders gathered bags directly from farmer homes.

Labor for AC manure cakes included: (1) Collection of fresh manure from the cattle shed and preparation of manure cakes by hand (young and elderly were often responsible for this step), (2) placement of cakes on the ground, fences, posts, and exterior walls to sun dry for approximately four days (weather dependent), (3) bagging in small sacks, and (4) sale. Sale-related labor was only included if farmers delivered bags to buyers.

Total annual manure-related labor in lowland households (n=101) was 254 h/yr (median = 200 h/yr) with SE ± 19.9 h/yr, and did not differ by commune. In a mixed model with household as a random effect, yearly labor reported for preparation of manure was highest for traded manure (cakes and broken-up) and lower for compost and fresh manure used for household crop production (Table 3). Labor associated with composted manure and fresh manure was in preparation for household crop fertilization use. Household explained 28% of residual variance in the model, suggesting significant within household variation in labor for different manure types. Total manure-related labor was positively correlated with number of cattle owned ($P=0.0004$). In a multivariate model predicting total manure-related labor, significant effects included number of cattle owned ($P<0.0001$) and commune ($P=0.0131$).

Table 3 Back-transformed least squares means (geometric means) and 95% CI for model estimating log-transformed annual hours invested in preparation and sale of cattle manure by manure type for lowland households (n=161).

Manure type	Manure related labor (h/yr)		
	Mean[a]	Lower 95% CI	Upper 95% CI
Broken-up	202 a	170	240
Cakes	153 a	117	199
Fresh	86.2 ab	22.6	329
Compost	52.7 b	44.9	61.8

[a] Means not connected by the same letter are significantly different.

Time invested in preparation and sale of manure (hours/m^3 dry manure) did not differ by commune or manure type (Table 4). Returns to labor were just over \$0.50/h (Table 4), which is in the same range as estimated off-farm labor wages (\$2 to \$5/day). Sale prices for broken-up manure were numerically higher than for manure cakes. Returns to labor were positively correlated with total cattle owned (P=0.0110, r=0.28) and total land owned and rented (P=0.0065, r=0.30). Returns to labor did not depend on total labor to prepare and sell manure.

Table 4 Labor requirements and returns to labor for the preparation and sale of cattle manure in Nhơn Khánh and An Chấn Communes.

n	Labor (hours/m^3)			Returns to labor (\$/h)		
	mean	median	SE	mean	median	SE
81	54.0	31.0	6.18	0.69	0.55	0.064

3.2.2 Manure collectors and traders

Manure collectors and traders consisted of lowland compost manure traders, small-scale lowland collectors, lowland traders and highland traders. All lowland collectors and traders identified during lowland farmer interviews were included in the sample (n=23). The group consisted of 13 in NK (5 traders and 8 small-scale collectors) and 10 in AC (3 traders, 5 small-scale collectors and 2 compost traders). Collectors and traders in NK and AC were each geographically distributed in four villages throughout their respective communes. Lowland trader households were predominately located on main commune roads. The trader dealing in highest annual volumes among those interviewed was located just outside AC on the main north-south highway, and collected manure from AC and other neighboring communes. Two traders in each commune identified manure trade as their primary occupation. One additional trader in each commune identified commercial transport of goods. Most lowland traders were truck owners (75%), and transported manure from lowland to sales locations. Small-scale collectors and remaining traders identified agriculture (farming and/or livestock) as their primary occupation.

Traders and collectors traded only in cattle manure, except for one NK trader who occasionally traded chicken manure. Traders and collectors in NK traded only in broken-up manure, while AC traders and small-scale collectors traded in both broken-up manure (51%) and manure cakes (49%). Most AC traders (two of three) mixed manure cakes with broken-up manure prior to resale to increase product attractiveness; buyers did not favor a high fraction of manure cakes. The remaining trader purchased only broken-up manure. Small-scale collectors purchased manure by the bag, and sold from their homes to lowland traders, highland traders, or highland farmers. Sales were both by the bag and per cubic meter, depending on buyer preference. Lowland traders generally purchased manure by the bag directly from farmers or from small-scale collectors and resold manure by the cubic meter. Two of 13 small-scale collectors sold only to highland farmers or highland traders. Most small-scale collectors (85% of 13 collectors) provided a collection service for traders by purchasing bagged manure from farmers and storing it for later pickup; six of these collectors also sold to highland traders. Lowland traders paid 1,000 to 1,500 VND/bag ($0.05/bag to $0.07/bag) for the collection service. Hired laborers were paid by traders at the same rate per bag for manure collection, loading and unloading or 15,000 VND/m^3 ($0.71/$m^3$). Most small-scale collectors stored manure at their home to prevent theft (85% of 13 collectors), although some traders managed collection points on the main road for daily pickup. Seven (of eight) traders reported oral contracts with small-scale collectors, with the understanding that traders set purchase prices (variable from month-to-month), provided sacks to small-scale collectors for manure collection, and established small-scale collector revenue/bag. Similarly, sacks were provided by all traders and collectors to farmers, who prepared and bagged the manure prior to sale.

Stored manure was covered with a tarp to prevent losses, especially in the event of rain. Traders and collectors reported a decrease in manure volume during storage, perhaps due to settling. One small-scale collector in NK removed rice straw and soil from the manure, which reduced volume available for sale and increased quality, according to traders and collectors (reported by 84% of 19 question respondents). Estimated losses from all these were low, between 0 and 5%. Collectors and traders evaluated manure quality (81% of 21 responding traders and collectors), preferring dry manure with minimal rice straw, soil, and other foreign materials, and a yellow or black color. These factors were not usually problematic, and had little to no impact on willingness to purchase or prices paid in transactions with lowland farmers.

Greatest challenges and risks reported by manure collectors and traders were price decline at the onset of the rainy season (four of 17 respondents) and competition among collectors and traders (including highland traders and farmers who come to the lowlands to buy manure) (three respondents), lack of means or difficulty transporting manure (three respondents), and volume and quality losses during rainfall events (eight respondents). One lowland trader noted that occasionally a translator was needed to facilitate highland sales transactions with ethnic minorities that did not speak Vietnamese.

Collectors and traders were asked if there have been times when they wanted to buy and sell manure, but could not. Among 12 traders responding affirmatively, 10 reported inability to operate in manure trade during the rainy season. Manure scarcity was also identified by two traders during periods of peak competition (April to June, when highland traders come to the lowlands to buy).

3.2.2.1 Lowland trader and collector transactions

Overall manure purchase price (n=48) and sale price (n=47) in lowland trader and collector-reported transactions was \$19.05/m^3 with SE ± \$0.28/m^3 and \$26.14/m^3 with SE ± \$1.11/m^3, respectively. Multivariate manure purchase price and sales price models were fitted separately (Table 5). Significant effects in the purchase price model included commune, trader type (small-scale lowland collector or lowland trader), manure type (broken-up, cakes or mixed), and transaction month. Traders and collectors purchased manure in AC at a significantly higher price than NK. Trader type also affected purchase price, with lowland traders offering a higher purchase price than small-scale lowland collectors. Transaction purchase price for broken-up manure or a mix of broken-up and caked manure was higher than manure cake transactions. Significant effects in the sale price model included trader type and transportation inclusion in transaction (Table 5). Lowland traders sold manure for significantly higher prices than small-scale lowland collectors. Sales transactions that included transportation to distant buyer destinations fetched a significantly higher price than those not including transportation (e.g., buyers or highland traders traveled to the lowlands to purchase manure directly from traders or collectors).

Mean transaction purchase (n=47) and sales volumes (n=47) were 8.83 m^3/transaction (median = 1.67 m^3/transaction) with SE ± 3.63 m^3/transaction and 29.5 m^3/transaction (median = 14.3 m^3/transaction) with SE ± 10.7 m^3/transaction, respectively. A multivariate model estimating transaction purchase volume indicated significantly higher volumes for lowland trader transactions than small-scale collector transactions (Table 6). Significantly higher volumes were also detected when collectors or traders provided transport from farmers or small-scale collectors to trader collection points. Sales volume model indicated significantly higher sales volumes for lowland trader transactions than small-scale lowland collectors (Table 6). Higher sales transaction volumes occurred for transactions of broken-up manure and mixed broken-up and caked manure than manure cakes alone.

Table 5 Least square means (LSM) and standard errors for lowland trader and collector purchase price model (left, n=48) and sale price model (right, n=47).

Effect[c]	Category	Purchase price[a] ($\$/m^3$)		Sale Price ($\$/m^3$)	
		LSM[b]	SE	LSM[b]	SE
Commune					
P<0.0001	AC	20.23 a	0.32		
Power=0.9998	NK	17.66 b	0.46		
Trader Type					
P=0.0003	Lowland trader	19.70 a	0.42		
Power=0.9743	Small-scale lowland collector	18.19 b	0.33		
Manure Type					
P=0.0039	Broken-up	19.90 a	0.22		
Power=0.8774	Mix	19.15 ab	0.81		
	Cakes	17.78 b	0.49		
Transport to buyer					
P<0.0001	Y			33.21 a	0.96
Power = 1.0	N			22.35 b	0.68
Trader Type					
P=0.0030	Lowland trader			29.91 a	0.70
Power=0.8661	Small-scale lowland collector			25.65 b	0.90

[a] Purchase price model contains transaction month continuous covariate (P<0.0001).
[b] Means not connected by the same letter are significantly different.
[c] Effect P values and power are indicated below effect name.

Table 6 Back-transformed least square means (geometric means) and 95% confidence intervals for log-transformed transaction purchase volume model (n=47) and log-transformed transaction sales volume model (n=47).

Effect[c]	Category	Purchase volume (m^3/transaction)			Sales Volume[a] (m^3/transaction)		
		LSM[b]	Lower 95% CI	Upper 95% CI	LSM[b]	Lower 95% CI	Upper 95% CI
Trader Type							
P=0.0007	Lowland trader	4.11 a	2.37	7.13			
Power=0.9452	Small-scale lowland collector	1.16 b	0.76	1.76			
Collector transports manure							
P=0.0029	Y	3.77 a	2.47	5.76			
Power = 0.8700	N	1.26 b	0.72	2.18			
Trader Type							
P<0.0001	Lowland trader				27.3 a	19.5	38.2
Power=1.0	Small-scale lowland collector				7.99 b	6.08	10.5
Manure Type							
P=0.0008	Mix				29.8 a	17.7	50.2
Power=0.9561	Broken-up				15.4 a	12.6	18.9
	Cakes				7.02 b	4.36	11.3

[a] Sales volume model also contains transaction month continuous covariate (P=0.0006).
[b] Means not connected by the same letter for each effect in each model are significantly different.
[c] Effect P values and power are indicated below effect name.

3.2.2.2 Annual manure purchases and sales for lowland traders and collectors

Lowland traders and collectors also reported manure purchase and sale prices and volumes on an estimated annual basis, and purchase prices reported by lowland traders in AC were significantly higher than prices indicated by small-scale lowland collectors in both communes (Table 7). Similarly, sales prices by lowland traders were significantly higher than sales prices by small-scale lowland collectors (Table 7). Annual manure trade volumes (n=21) were significantly higher for lowland traders than small-scale lowland collectors by a factor of 12.6:1 (Table 8). Annual volumes did not differ by commune for the two lowland trader types.

Table 7 Least square means and standard errors for overall purchase price model (left, n=21) and overall sales price model (right, n=20) by lowland traders and collectors.

Trader Type (commune)[b]	Purchase Price ($/m^3)		Sale Price ($/m^3)	
P=0.0030 Power=0.9247	LSM[a]	SE	LSM[a]	SE
Lowland trader (AC)[c]	22.10 a	0.93		
Lowland trader (NK)[c]	19.20 ab	0.72		
Small-scale lowland collector (AC)[c]	18.29 b	0.72		
Small-scale lowland collector (NK)[c]	16.96 b	0.57		
Trader Type[b]				
P<0.0001 Power=1.0				
Lowland trader			30.68 a	1.34
Small-scale lowland collector			19.67 b	1.10

[a] Means not connected by the same letter are significantly different.
[b] Effect P values and power are indicated below effect name.
[c] AC = An Chấn Commune; NK = Nhơn Khánh Commune

Table 8 Back-transformed least square means (geometric means) and 95% confidence intervals for annual collector and trader log-transformed volume model (n=21).

Trader Type[b] n=21, P=0<0.0001 Power=0.9999	Volume (m^3/yr) LSM[a]	Lower 95% CI	Upper 95% CI
Lowland trader	3,350 a	1,681	6,674
Small-scale lowland collector	266 b	155	456

[a] Means not connected by the same letter are significantly different.
[b] Effect P value and power are indicated below effect name.

Manure revenues, labor costs, and transportation costs varied by trader type (Table 9a). Only four small-scale lowland collectors reported labor costs, because many collectors do not hire labor. Similarly, with the exception of lowland traders in NK, other traders and collectors did not report transportation costs. Average net incomes, calculated as *revenue - hired labor costs - transportation costs* for individual traders, were significantly higher for lowland traders than small-scale lowland collectors and compost traders (Table 9b).

Table 9a Descriptive statistics for lowland trader and collector revenue, manure-related labor costs, and manure-related transport costs.

			NK[a] Lowland trader	AC[a] Lowland trader	NK[a] Small-scale lowland collector	AC[a] Small-scale lowland collector	AC[a] Compost trader
Revenue	$/yr	*n*	5	3	7	5	2
		Mean	44,039	41,578	931	505	86
		Median	46,396	25,454	743	267	86
		SD	35,403	46,506	828	574	81
Hired labor costs	$/yr	*n*	5	3	3	1	
		Mean	3,058	7,223	347	714	
		Median	2,619	2,880	371	714	
		SD	2,574	8,188	229		
Transportation costs	$/yr	*n*	5	1	1		
		Mean	19,795	14,141	102		
		Median	16,857	14,141	102		
		SD	19,801				

[a] AC = An Chấn Commune; NK = Nhơn Khánh Commune

Table 9b Back-transformed least square means (geometric means) and 95% confidence intervals for log-transformed trader net income from manure trade model (n=22).

Trader Type (commune) n=22, P=0<0.0001 Power=1.0		LSM[a]	Net income $/trader/yr Lower 95% CI	Upper 95% CI
NK[b]	Lowland trader	16,953 a	5,662	50,758
AC[b]	Lowland trader	11,949 a	2,901	49,223
NK[b]	Small-scale lowland collector	484 b	192	1,223
AC[b]	Small-scale lowland collector	226 b	76	677
AC[b]	Compost trader	64 b	11	362

[a] Means not connected by the same letter are significantly different.
[b] AC = An Chấn Commune; NK = Nhơn Khánh Commune

3.2.2.3 Lowland trader and collector labor and returns to labor

Labor (time) included reported family labor (unpaid) and hired labor. Small-scale lowland collectors in AC did not hire laborers. Four small-scale lowland collectors in NK hired a team of 6 laborers to assist only with loading transporter trucks with manure. All lowland traders hired labor to assist with collection, loading, unloading and transport (for traders providing transport to distant buyers).

Small-scale collector labor consisted of the following steps: (1) collection of bagged manure from farmers, (2) unloading and storage at a collection point near the house, and, (3) sale to lowland or highland traders who picked manure up at collection points. Some transactions included labor to load trucks. One lowland collector removed rice straw and other foreign material from product to increase value.

Lowland trader labor consisted of: (1) collection from small-scale collectors and farmers, (2) unloading and storage at a collection point close to the house, (3) loading on a personal truck for transport to highlands or on highland trader truck, (4) transport to highlands to sell, and, (5) sale of manure at highland destinations. Traders owning trucks and transporting manure to the highlands to sell sometimes brought highland products back to the lowlands to sell during the return journey to increase commercial travel benefits.

Time invested in trade of one cubic meter of manure (n=20) did not depend on commune or trader type, and was 2.67 h/m^3 (median = 2.18 h/m^3) with SE ± 0.510 h/m^3. Annual labor (hours) invested in manure trade activities was significantly higher for lowland traders than lowland collectors and compost traders (Table 10). Returns to labor, calculated as net income divided by annual manure trade labor, were significantly higher for NK traders than AC traders (Table 11). Returns did not depend on trader type.

Table 10 Back-transformed least square means (geometric means) and 95% confidence intervals for log-transformed annual manure trade labor model (n=22).

Trader Type (commune)[b] P=<0.0001 Power=1.0		LSM[a]	Labor (hours/yr) Lower 95% CI	Upper 95% CI
AC[c]	Lowland trader	10,489 a	3,659	30,066
NK[c]	Lowland trader	6,776 a	2,997	15,320
NK[c]	Small-scale lowland collector	475 b	238	946
AC[c]	Small-scale lowland collector	417 b	184	942
AC[c]	Compost trader	66 b	18.1	239

[a] Means not connected by the same letter are significantly different.
[b] Effect P values and power are indicated below effect name.
[c] AC = An Chấn Commune; NK = Nhơn Khánh Commune

Table 11 Least square means and standard errors for returns to trader labor (n=22).

Commune[b] P=0.0362 Power=0.5705	Returns to labor ($/h) LSM[a]	SE
NK[c]	2.15 a	0.33
AC[c]	1.05 b	0.36

[a] Means not connected by the same letter are significantly different.
[b] Effect P values and power are indicated below effect name.
[c] AC = An Chấn Commune; NK = Nhơn Khánh Commune.

3.2.2.4 Highland traders

Four highland traders were interviewed in Gia Lai Province in Chư Sê (n=2), Đăk Đoa (n=1), and Ayun Pa (n=1) Districts. The Ayun Pa trader was the only trader operating in the district. The Đăk Đoa trader reported 10 other traders in the district. All traders purchased broken-up manure using pre-arranged oral agreements with sellers. Traders discussed the quality, quantity and price of manure with potential sellers by phone before agreeing to a transaction. Most transactions were with lowland traders. Two highland traders also reported purchases from lowland farmers and small-scale lowland collectors. Highland traders emphasized the need to maintain a close relationship with lowland traders and transporters to ensure successful transactions, and underscored the importance of checking manure quality prior to finalizing purchase transactions. High quality manure was reportedly yellow in color, dried on the ground and broken-up (not in cakes), and contained minimal rice straw and sand. Poor manure quality could result in transaction cancellation (reported by one trader).

Three of four traders owned a truck that was used for hauling manure. The Chư Sê trader without a truck worked with lowland traders who delivered manure to a distribution point near the highland trader's household. Deliveries were made between 2:00 am and 6:00 am daily. From the distribution point, it was resold to highland transporters or farmers who visited the distribution point to complete the transaction. The second Chư Sê trader and the Đăk Đoa trader purchased manure in the Bình Định lowlands and transported it to the highlands in their truck for resale to highland farmers, often after a brief storage delay at a distribution point. The sale of manure from highland traders to farmers generally included delivery for the three traders with trucks. Storage was typically in a covered pile at the distribution point when manure was unloaded prior to resale. Loaded trucks were covered with a tarp during the transport process to prevent manure loss. Only the Đăk Đoa trader reported problems with manure transport losses. Traders sold to coffee and pepper farmers, with all sales to farmers from the Đăk Đoa trader and one Chư Sê trader. The other Chư Sê trader and the Ayun Pa trader sold most manure to highland transporters (80% and 95%, respectively).

Traders reported seasonal fluctuations in manure price, which they attributed to changes in demand. Highland farmers apply manure at the beginning of the rainy season and must purchase manure one to three months in advance to prepare it for application (described below). Thus, prices are highest after coffee and pepper harvest until one month before the rainy season (December to May), and decrease with the onset of the rainy season. This report coincided with lowland farmer observations (Figure 3). Highland traders observed a rapid decline in price with rainy season onset, which was frustrating from trader viewpoints as price changes were sudden with inconsistent year-to-year timing (climate change impacting onset of rainy season), and occasional high remaining inventories that had to be sold at lower prices or stored.

Challenges reported by highland traders included an inability to purchase manure in the volumes desired during periods of high demand, because of competition from other highland and lowland traders, and inability to sell manure during the rainy season (manure is stored). Manure volume losses from mineralization were reported as a high business risk for manure not sold prior to the rainy season. However, traders agreed that these risks (costs) did not offset the economic benefits of manure trade.

Highland trader transactions took place in May, July, and August 2013 (Table 12). Net purchase prices included transport and loading fees, and net sales prices included deductions for transport and loading fees. Purchase and sale net dollar amounts per transaction also accounted for these transport and loading fees, which were incurred independently for the purchase and sale processes. Purchase volumes were numerically higher than sales volumes. Gross mark up in highland trader-reported transactions was 21%.

Table 12 Descriptive statistics for highland trader-reported transactions (purchases and sales) including transaction volume, price, net price (adjusted for transport and loading fees), and $ per transaction net (adjusted for transport and loading fees).

Transaction type	n	Volume (m^3/transaction)			Price ($/$m^3$)			Net price ($/$m^3$)			Transaction net ($/transaction)		
		Mean	Med	SD	Mean	Med	SD	Mean	Med	SD	Mean	Med	SD
Purchase	5	30.5	22.0	18.1	21.71	23.81	6.91	23.57	23.81	10.26	706	548	480
Sale	5	12.3	14.3	3.95	29.43	26.67	6.46	28.57	25.71	6.06	358	381	153

Highland traders reported annual manure trade volumes and average purchase and sale prices (Table 13). The Chư Sê and Ayun Pa traders selling mostly to transporters traded in much higher annual volumes (mean = 24,975 m^3/yr) than the other traders (mean=1,225 m^3/yr). Annual purchase and sale amounts ($) were calculated for individual traders by deducting loading fees. This impacted net incomes for high volume traders, because they covered the cost of loading transporter trucks. Thus, net incomes for traders dealing in high volume (mean=$15,411/yr) were close to net incomes for low-volume traders (mean = $12,232/yr). Low-volume traders did not report transport or labor costs, thus, net incomes could be inflated.

Table 13 Descriptive statistics for annual manure trade volumes, average prices, and net incomes by highland traders, as well as price and annual dollar amounts for purchases and sales.

	n	Volume (m^3/yr)			Price ($/$m^3$)			Annual amount ($)		
		Mean[a]	Median	SD	Mean[a]	Median	SD	Mean[a]	Median	SD
Purchase	4	13,100	10,950	14,214	24.64	23.81	3.95	357,179	317,214	388,525
Sale	4	13,100	10,950	14,214	30.54	30.95	1.72	384,652	338,929	407,929
Net Income ($/yr)								**13,821**	**12,232**	**5,274**

[a] Values reported are averages per trader.

3.2.3 End users

End users included Central Highland pepper and coffee farmers, Bình Thuận dragon fruit farmers and rubber companies in the Central Highlands.

3.2.3.1 Highland pepper and coffee farmers and Bình Thuận dragon fruit farmers

Highland farmers (n=50) in five communes (12 villages) reported cultivation of 1.73 ha with SD ± 1.29 ha. Dragon fruit farmers (n=20) interviewed were located in two communes (7 villages) in Bình Thuận Province, and reported cultivation of 0.749 ha with SD ± 0.529 ha.

Predominant crops cultivated by highland farmers were black pepper (78% of highland farmers) and coffee (70%). Less often cultivated crops included rice (14%), corn (6%), cashew (4%), rubber (2%) and sugar cane (2%). Highland farmers purchased cattle manure for use as an organic soil amendment for pepper and coffee lands. Farmers applied manure to pepper yearly, approximately one month post-harvest at the beginning of the highland rainy season (April to May). Applications to coffee were less frequent, every one to four years (mode = two years) at the beginning of the rainy season (April to May). Predominant crops cultivated by Bình Thuận farmers were dragon fruit (95% of respondents) and rice (25%). Bình Thuận farmers purchased manure to use as an organic amendment on land established in dragon fruit. Farmers reported higher yields with manure use. Most farmers applied manure two times per yr, once between March and May and again between August and October.

Greatest nutrient management challenges in Bình Thuận included soil quality (25% of Bình Thuận farmers) and flooding during the rainy season (30%). Highland challenges included poor soil fertility (34% of highland farmers), flooding and erosion during the rainy season (12%), pepper bacterial diseases (8%), drought (4%), and lack of capital to buy cattle manure. Low soil pH, soil organic matter and soil fertility reportedly occurred if composted cattle manure applications were not possible, which may drive the high seasonal demand for cattle manure by destination farmers.

Manure purchase transactions and preparation in the highlands consisted of first calling Phú Yên, Bình Định, or highland traders, or travel to the main road. During the height of the manure trade season, many traders travel to the highlands and park their trucks on the main road. Farmers seeking manure visit the trucks to inspect the manure for quality and negotiate a price. Manure is then transported to the farm for delivery and unloading. Alternatively, some farmers who have trucks travel to the lowlands to purchase manure. Manure is composted on the farm for one to two months prior to use according to the recipe below. Crop application consists of digging a trench around the pepper or coffee tree and filling the trench with composted manure.

Manure purchase transactions and preparation for use in Bình Thuận consisted of first calling local traders, Phú Yên traders, or southeast coastal traders to inquire about manure availability and quality. Traders then transport manure to the farm and manure quality is evaluated prior to transaction finalization. Manure is composted by farmers at the house for one to two months, and then applied by digging a trench around the base of each dragon fruit tree, filling the trench with composted manure, and covering it with rice straw to maintain soil humidity. The purchase price of a truckload of rice straw is $90 to $150.

Only six highland farmers (12%) and one Bình Thuận farmer (5%) applied manure directly as an organic soil amendment without composting. Highland farmers described preparation of composted manure prior to application according to a variation of the following recipe. Urea fertilizer (3 kg) and potassium (2 kg) are dissolved in 200 L of water. Dry manure (200 kg) is mixed with coffee pulp (100 kg). The compost pile is formed with 30-cm layers of manure and coffee pulp. Each layer is moistened with the urea-potassium solution, along with probiotics (1 to 2 kg for the batch) and phosphorus (5 kg P_2O_5/batch). The compost pile usually reaches about one to 1.2-m high and one to 1.5-m base diameter. The pile is covered with a tarp and remoistened with water every three days. The pile is mixed after 15 days and covered again for one month. It is then ready for crop application or storage until crop application. Another ingredient commonly used in highland compost is lime.

Farmers reported composted manure application on 92% of pepper fields, 63% of coffee fields, and 100% of dragon fruit fields. Crop density (trees/ha) was positively correlated with compost application rate (r=0.45). A multivariate model was selected with composted cattle manure application rate response and fixed effects of crop (pepper, coffee, dragon fruit) and crop density (trees/ha) (Table 14). Establishment regions varied by crop, with respondents cultivating dragon fruit in Bình Thuận Province and coffee and pepper respondents in highland provinces of Đắk

Lắk and Gia Lai. Significantly higher compost application rates reported for dragon fruit could be attributed to regional management differences rather than crop differences. Other fertilizers commonly used in pepper and coffee cultivation include phân vi sinh (a commercial organic amendment, described below), urea, NPK blends, ammonium sulfate, and fertilizers containing potassium and phosphorus (e.g., muriate of potash (KCl) and diammonium phosphate). Composted manure and fertilizer application rates indicated average rates and variation for all crops (Appendix 1 Table A2).

Table 14 Back-transformed least square means (geometric means) and 95% CIs for composted manure application rate (n=90) in a model predicting log-transformed farmer-reported application rate.

Crop	Compost application rate (Mg DM/ha per yr)[a]		
	LSM[b]	Lower 95% CI	Upper 95% CI
Dragon fruit	10.9 a	9.24	12.9
Coffee	7.03 b	6.01	8.21
Pepper	4.43 c	3.96	4.96

[a] Model effects included crop ($P<0.0001$, Power=1.0) and density (trees/ha) ($P<0.0001$).
[b] Means not connected by the same letter are significantly different ($P \leq 0.05$).

Farmers were asked what could be done to add additional value to manure. Composting as per their guidelines prior to delivery would increase value (100% of 59 responding farmers). Common compost requests were with probiotics (39%), with phosphorus (14%), with lime (8%), with urea and phosphorus (8%), and compost without requests for addition of specific ingredients (15%). However, farmers did not trust traders to do it properly. Reported manure alternatives included phân vi sinh (an organic amendment, described below) (73% of 67 responding farmers), coffee pulp (16%) and household cattle manure (7%). Other alternatives mentioned were quail manure (1 farmer), rice straw (1 farmer), sugar cane byproducts (2 farmers) and chemical fertilizer (4 farmers).

Farmers with available cash prefer to buy inexpensive manure in August or December, and compost and store it for later us, but most small farmers do not have cash available at that time and must wait until post-harvest to purchase manure when prices and demand in the highlands are high, which suggests a role for credit to purchase manure in the region.

3.2.3.1.1 Highland and Bình Thuận farmer manure purchase transactions

Manure purchase transactions (n=88) were reported by 70 farmers in highland and Bình Thuận locations. Overall manure purchase price in transactions was $36.15/m^3 with SE ± $0.82/m^3. In a multivariate model, purchase price was affected by buyer location (highland or BT), and by the interaction between seller location and manure form (bag or m^3), suggesting that the impact of seller location on price depends on manure form (Table 15). All transactions were with traders, and price included unloading labor. Transaction month did not impact amount, volume or price. Purchase price for highland farmers was significantly higher than Bình Thuận dragon fruit farmers. Bagged manure sold for a numerically higher price than non-bagged manure from all seller locations except the southeast lowlands. Bagged manure from BD was significantly more expensive than non-bagged product.

Mean transaction volume (n=88) was 22.2 m^3/transaction (median = 16.7 m^3/transaction) with SE ± 2.53 m^3/transaction. In a multivariate model, manure class, buyer province and their interaction were significant (Table 16). Interaction effect indicated that non-bagged transaction purchase volumes were higher than bagged volumes, and transaction volumes for buyers in Gia Lai and Bình Thuận tended to be higher than Đắk Lắk.

Mean transaction amount (n=88) was $778 (median = $490) with SE ± $95.1. In a multivariate model, significant effects included manure class and buyer province (Table 16). Transaction amounts were higher for non-bagged manure than bagged manure, mostly due to higher volumes. Transaction costs were significantly higher for purchase transactions in Bình Thuận and Gia Lai than Đắk Lắk, also consistent with reported larger transaction volumes in these regions.

Transaction challenges identified by farmers included difficulty in evaluating quality (45% of 31 farmers reporting challenges) and high manure prices, lack of capital to purchase enough manure, or inability to buy manure when price is lower due to cash flow problems (45%). Two farmers reported that some traders permit partial payment, with the balance due post-harvest. Farmers attributed high prices to demand early in the year when cash is available from recent harvests. Timing is also favorable for composting prior to application on the current year crop.

Table 15 Least square means and standard errors for transaction price model (n=88) for highland pepper and coffee farmers and Bình Thuận dragon fruit farmers.

Effect[b]	Manure class, seller location	Manure price ($/m^3)	
		LS Mean[a]	SE
Manure class x Seller location P<0.0001, Power=1.0	Bagged, BD[c]	41.09 a	1.78
	Bagged, highland	39.63 ab	2.61
	Bagged, PY[c]	37.25 ab	0.86
	Non-bagged, southeast lowlands	35.57 abc	2.06
	Non-bagged, PY[c]	35.44 ab	0.98
	Non-bagged, highland	32.19 bcd	1.68
	Non-bagged, BD[c]	27.74 cd	1.34
	Bagged, southeast lowlands	23.10 d	2.28
Farmer (buyer) location P<0.0001, Power=1.0	Highland	38.81 a	0.76
	BT[c]	29.19 b	1.03

[a] Means not connected by the same letter are significantly different.
[b] Effect P values and power are indicated below effect name.
[c] Bình Định Province = BD, Phú Yên Province = PY, Bình Thuận Province = BT

Table 16 Least square means (geometric means) and 95% confidence intervals for log-transformed manure transaction volume model (left, n=88) and log-transformed transaction amount model (right, n=88) for highland pepper and coffee farmers and Bình Thuận dragon fruit farmers.

Effect[b]	Manure class, buyer province[c]	Manure volume (m^3/transaction)			Transaction amount ($/transaction)		
		LS mean[a]	Lower 95% CI	Upper 95% CI	LS mean[a]	Lower 95% CI	Upper 95% CI
Manure Class x buyer province P=0.0343, Power=0.6402	Non-bagged, GL	27.6 a	20.8	36.7			
	Non-bagged, BT	25.3 ab	17.2	37.4			
	Bagged, BT	13.3 b	8.90	19.9			
	Non-bagged, DL	7.73 bc	3.63	16.4			
	Bagged, GL	5.94 c	4.24	8.33			
	Bagged, DL	3.72 c	2.10	6.59			
Manure class P<0.0001, Power=1.0	Non-bagged				680 a	523	883
	Bagged				235 b	182	304
Province P=0.0118, Power=0.7728	BT				531 a	398	708
	GL				507 a	404	635
	DL				238 b	148	380

[a] Means not connected by the same letter for each model are significantly different.
[b] Effect P values and power are indicated below effect name.
[c] Gia Lai Province = GL, Bình Thuận Province = BT, Đắk Lắk Province = DL.

Farmers were skeptical of manure quality, and one farmer believed that traders may add other components to manure to increase volume (e.g., soil, rice straw). Thus, quality evaluation often occurred during the transaction process, and substandard product could result in transaction cancellation and reloading of any unloaded manure. This occurrence was not common, but possible. Common factors decreasing quality included sand, excess rice straw, and other non-manure components. Transactions were contingent on high quality manure, thus most farmers (67%) did not pay a price premium for high quality manure. A minority of farmers (26%) were still willing to complete transactions for manure with quality deficiencies if the seller would drop the price. Farmers reporting a more positive experience recommended establishing a relationship with traders and local farmers to help ensure high quality manure and timely delivery. Transactions conditional on manure quality inspection were recommended. Highland farmers generally believed that manure quality was better for manure purchased from local highland cattle farmers than lowland manure.

3.2.3.1.2 Annual manure purchases by end users

Annual manure purchases were made by coffee and pepper farmers in highland provinces (Đắk Lắk and Gia Lai) and dragon fruit farmers in lowland Bình Thuận province. Purchase prices were higher in highland districts than in Bình Thuận (Table 17) in a multivariate model containing binary location (highland or southeast), district nested in location, and a categorical variable for manure purchase category (by the bag, truck, or both). Regional purchase price differences may be largely due to the timing of transactions, and supply and demand patterns in the highlands. Manure purchased by the bag was significantly more expensive than manure sold by the truckload. Prices reportedly dropped in the highlands as the rainy season began in July or August. Bình Thuận manure sales from south-central coastal regions commenced around the same time, and lower prices persisted for these sales to dragon fruit farmers.

Household land area in pepper, coffee and dragon fruit (n=70) was positively correlated with total annual manure purchase volume (r=0.53) and annual manure expenditures (r=0.59). Multivariate models estimating square root-transformed purchase volume or square root-transformed annual expenditures contained significant effects of province and land area in pepper, coffee, and dragon fruit (Table 18). Volumes and expenditures differed among the three provinces, and decreased in order from Bình Thuận to Gia Lai to Đắk Lắk.

Table 17 Least square means and standard errors for manure purchase price model (n=70) by farmers interviewed in manure purchasing districts.

Effect[b]		Manure price ($/m^3) LS Mean[a]	SE
District[c]			
P = 0.0014	Chư Pưh, GL, highland	44.48 a	2.14
Power = 0.9589	Chư Prông, GL, highland	42.19 ab	2.15
	Buôn Hồ, DL, highland	41.75 ab	2.20
	Chư Sê, GL, highland	37.05 abc	2.14
	Đăk Đoa, GL, highland	34.41 bcd	2.26
	Hàm Thuận Bắc, BT, southeast coast	31.50 cd	1.87
	Hàm Thuận Nam, BT, southeast coast	27.32 d	1.98
Sale type			
P = 0.0479	Bag	36.91 a	1.15
Power = 0.5903	Both	34.27 ab	3.38
	Truck	32.90 b	1.09

[a] Means not connected by the same letter are significantly different for each effect.
[b] Effect P values and power are indicated below effect name.
[c] District, Province (Gia Lai Province = GL, Bình Thuận Province = BT, Đắk Lắk Province = DL), highland or southeast coast.

Table 18 Least square means (geometric means) and 95% confidence intervals for square root-transformed annual manure purchase volume model (n=70) and square root-transformed annual manure expenditures model (n=70) as reported by farmers purchasing manure.

Province	Manure volume (m^3/yr)[b] P < 0.0001, Power = 0.9995			Manure expenditures ($/yr)[b] P < 0.0001, Power = 0.9936		
	LS mean[a]	Lower 95% CI	Upper 95% CI	LS mean[a]	Lower 95% CI	Upper 95% CI
Bình Thuận	45.5 a	34.2	58.5	1403 a	1,040	1,819
Gia Lai	21.6 b	16.3	27.7	799 b	612	1,012
Đắk Lắk	6.13 c	1.67	13.4	265 c	89	535

[a] Means not connected by the same letter are significantly different for each model.
[b] Effect P values and power are indicated below effect name.

3.2.3.2 End users: rubber companies

Two rubber companies were interviewed, one in Gia Lai Province (8,400 ha in rubber), and one by phone in Khánh Hòa Province (300 ha in rubber) (directly south of Phú Yên). The Gia Lai company purchased manure as the foundation ingredient in production of phân vi sinh under the trademark “Long Vân”. Phân vi sinh, a commercial organic fertilizer with microorganisms, is used as a soil amendment for rubber, coffee, pepper and dragon fruit plants. Many formulations exist but a representative product label contained 15% organic matter from manure, 1.5% P_2O_5, 2.5% humic acid, 1% Ca, 0.5% Mg, and 0.3% S (concentration of other nutrients was not indicated). Bacteria were also added including aspergillus, azotobacter, and bacillus (1×10^6 CFU/g of each microorganism).

Manure purchases were made from traders in Phú Yên (60%) and Gia Lai (40%) Provinces, and transactions were completed as independent contracts. Contract payments were made after company associates verified agreed volume and manure quality in the transaction. Manure quality was considered higher for manure in broken-up form (cakes are considered of lower quality) and manure containing < 5% rice straw. The company sometimes tests manure samples for OM, N, P, and K concentrations. Contracts can be terminated and the transaction cancelled if > 50% of a load is manure cakes. A cattle manure substitute for phân vi sinh production is peat, although phân vi sinh quality reportedly declines when non-manure substitutes are used. The company prefers to purchase manure when local farmer demand is low (later in the year), because bulk purchase prices are lower. Two manure purchase transactions, reported in 2013 from a trader in AC commune, Phú Yên, averaged 1,750 m^3/transaction at a purchase price of \$31.43/m^3. The company normally purchases manure once in January or February and again in July for an annual total of 4,000 to 5,000 m^3. Purchase prices range between \$28.57/m^3 \$34.29/m^3, with highest prices early in the year. The second purchase is used to make phân vi sinh for the following year.

The Khánh Hòa company purchased manure on a contractual basis from a trader in Phú Yên for use as a soil amendment in establishment year rubber trees. Prior to application, manure was composted for two months. Quality, a criterion for contract termination, was inspected. A single 2013 purchase transaction was reported in October from a Phú Yên trader for 200 m^3 at a price of \$30.95/m^3. The trader provided transportation (200 km, one way), which was included in purchase price.

Both companies fertilized with phân vi sinh at an average rate of 1.8 kg/tree per yr (810 kg/ha). The Khánh Hòa company fertilized establishment year rubber trees with 10 kg composted manure/tree (4.5 Mg/ha) and 0.3 kg phân vi sinh (135 kg/ha). Additional annual fertilizer rates used for established rubber trees were 0.35 kg urea/tree (157.5 kg/ha), 0.35 kg NPK/tree (157.5 kg/ha), 0.35 kg K_20/tree (157.5 kg/ha), and 0.45 kg P_2O_5/tree (202.5 kg/ha).

3.3 Overall quantitative comparison of value chain actors

Manure purchase and sale prices in transactions were combined in a single model to demonstrate price progression through value chain participants, and to provide qualitative evidence for value addition at different steps (Figure 4). The difference between purchase and sale prices was higher for lowland traders than other intermediaries due to manure transport over long distances (100 to 400 km). Highland pepper and coffee farmers purchased manure for a significantly higher price than rubber companies and dragon fruit farmers.

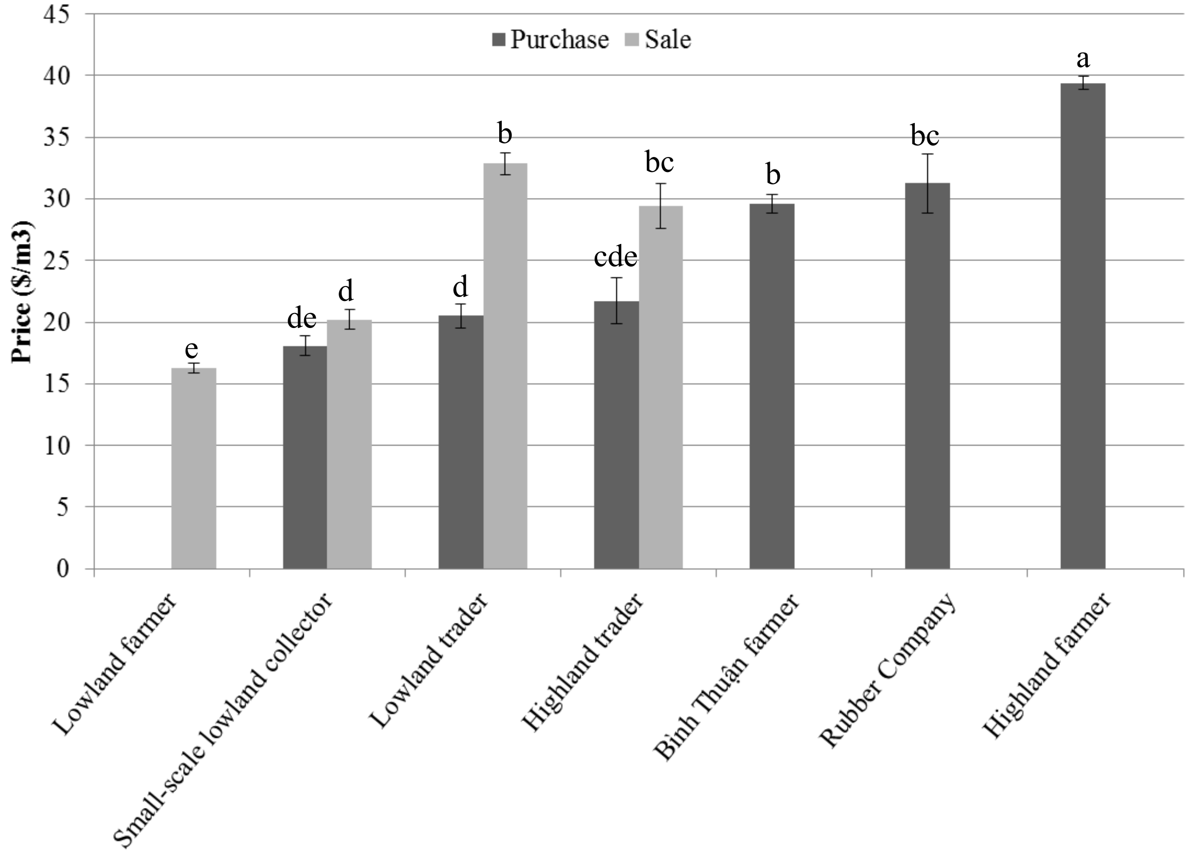

Figure 4 Purchase and sale price least squares means for manure value chain participants through the value chain. Error bars represent 1 SE of the mean. Means connected by the same letter did not differ. Bình Thuận = BT.

Lowland traders earned significantly more income per hour than small-scale lowland collectors and lowland farmers (Table 19), primarily due to the cost of transportation over long distances (100 to 400 km) to manure trade destinations. Small-scale lowland collectors also received significantly greater returns than lowland farmers. Labor invested in manure trade (h/m^3) was significantly higher for lowland farmers than collectors or traders.

Table 19 Least square means (LSM) and standard errors for returns to labor model ($P < 0.0001$, Power = 0.9989) and back-transformed least squares means (geometric means) and 95% confidence intervals for log-transformed labor use model ($P < 0.0001$, Power = 1.0) and log-transformed total labor model ($P < 0.0001$, Power = 1.0) by actor (n=101 for each model).

Actor	Returns to labor ($/h)		Labor (h/m³)			Total labor (h/yr)		
	LSM[a]	SE	LSM[a]	Lower 95% CI	Upper 95% CI	LSM[a]	Lower 95% CI	Upper 95% CI
Lowland trader	2.25 a	0.26	2.38 b	1.30	4.38	7,983 a	4,668	13,650
Small-scale lowland collector	1.36 b	0.21	1.83 b	1.12	3.01	450 b	290	697
Lowland Compost trader	0.99 abc	0.52	-	-	-	65.7 c	22.5	192
Lowland farmer	0.69 c	0.08	36.0 a	29.7	43.6	192 c	162	227

[a] Means in each labor column not connected by the same letter are significantly different.

Manure net incomes for manure value chain participants that sell manure were also combined for comparison (Table 20). Manure trader net incomes were significantly higher than small-scale lowland collectors, lowland farmers, and compost traders. Small-scale lowland collector net incomes were significantly higher than lowland farmer net incomes.

Table 20 Least squared means (geometric means) and 95% confidence intervals for log-transformed net income from cattle manure model for manure value chain participants (n=103, $P<0.0001$, Power = 1).

Actor	Net income ($/yr)		
	LSM[a]	Lower 95% CI	Upper 95% CI
Lowland trader	14,869 a	8,296	26,650
Highland trader	13,140 a	5,757	29,992
Small-scale lowland collector	353 b	219	568
Lowland Farmer	92.2 c	76.8	111
Lowland compost trader	63.9 bc	19.9	205

[a] Means not connected by the same letter are significantly different.

3.4 Fertilizer equivalence

We assumed 350 kg manure/m^3 based on farmer and collector reports. Average DM content of sold (dry) manure (89%), N concentration (12.7 g/kg DM) and P_2O_5 concentration (5.9 g/kg DM) were used with average transaction price to calculate fertilizer equivalencies. Price of N in manure sold from farmers to collectors and traders was \$4.11/kg N based on manure transaction rates or \$4.40/kg N for annual rates, which was substantially more expensive per N unit than chemical urea (46:0:0) (\$1.21/kg N, 2013 rates). Price of P_2O_5 in manure sold was \$8.85/kg P_2O_5 for transactions or \$9.47/kg P_2O_5 for annual rates, and was also much more expensive per P_2O_5 unit than chemical P_2O_5 in diammonium phosphate (18:46:0; N:P_2O_5:K_2O) (\$1.97/kg P_2O_5, 2013 rates). Large differences in N and P fertilizer value equivalents indicate that something other than these nutrients is valued in manure. Farmers were unaware of nutrient concentrations in manure, but believed its properties as an organic soil amendment were more important than potential nutrient supply.

Equivalency rates for manure sold to highland farmers by traders were even higher when compared with economical N and P fertilizer sources (6.5 to 7.5 times the cost of N in urea and 8.5 to 10 times the cost of P_2O_5 in diammonium phosphate), due to higher sales prices. A more reasonable highland comparison is with OM in commercial phân vi sinh, a common manure substitute that is made from cattle manure and other additives by some rubber companies (available commercial chemical formulation given above). Organic matter concentration in available commercial phân vi sinh ranged from 13% to 22%. A representative commercial phân vi sinh contained 15% OM, and was priced at \$0.21/kg (2013 rates, pre-tax). Thus, the cost of OM in phân vi sinh was \$1.43/kg OM. The cost of OM in manure sold to highland farmers and rubber companies was \$2.07/kg OM and \$1.78/kg OM, respectively. Thus, the cost of OM in manure was 1.2 to 1.5 times the price of OM in this specific phân vi sinh. Farmers reported that cattle manure was strongly preferred to commercial phân vi sinh, which combined with high demand for manure and high prices early in the year, increased manure value well above the equivalent price of chemical constituents in alternative commercial products.

4. Discussion

Over 65% of Vietnam's population resides in rural areas and 60% of the labor force is employed in agriculture, generating approximately 20% of gross domestic product (FAO, 2014). The historical and cultural importance of agriculture influences the high proportion of smallholder farmers and agricultural laborers. Farmers in this study, and perhaps in other areas of Vietnam, place high importance and value on endogenous sources of organic matter such as livestock manure. Active informal manure trade exists between south-central coastal Vietnam and the Central Highlands and southeast lowlands, as revealed in this study.

Barriers to smallholder market entry for value-added agricultural products such as high transactions costs (Markelova *et al.*, 2009) do not constrain smallholder participation in manure trade due to the presence of local collectors and traders, who are often farmers themselves. Furthermore, requirements to produce manure product are few and resources readily available, including animals (cattle), labor to collect, dry, and bag manure, and sacks, which are provided by traders. Moreover, the structure of informal cattle manure value chains in the region favors participation by smallholder lowland cattle farmers, because they are one of the few potential sources of livestock manure to supply highland markets in quantities demanded. Currently, lowland farmers can sell as much or as little manure as they desire. The only constraints to chain operation are difficulty drying manure, low highland demand, and low sales prices during the rainy season.

4.1 Value addition

Value was added to manure due to changes in what are called place utility, time utility, and form utility via drying, bagging, collection, transport, and composting prior to highland application. Greatest changes in manure product value may occur between collector and trader acquisition of manure and sale to destination buyers ($10 to $20 USD change per cubic meter). Changes also occur between animal excretion and farmer sale (at a mean price of $\$16/m^3$), although an unknown proportion of cattle production costs could be assigned to manure production and to the opportunity cost of manure use to fertilize crops on farm. Farmers added most form utility via production of dry manure product by gathering excreted manure, drying it in cakes or broken-up form, and bagging it for sale, thus transforming animal byproduct into a valuable marketable source of organic matter.

Collectors and traders constitute the essential value chain linkages between lowland cattle suppliers and distant destinations, and add value to manure product due to changes in place utility, time utility, and form utility. Services provided include adding place utility by manure aggregation (accumulating small quantities of lowland manure from spatially-dispersed farms) and transfer to destinations. This simplifies market entry for smallholder manure sellers by decreasing or removing distance to market barriers and reducing associated labor for sellers. It also simplifies manure purchase for destination buyers by providing a direct point of contact for highland buyers. The change in value between trader acquisition and end user purchase can be attributed primarily to changes in place utility via transport of product demanded by users over distances (100 to 400 km).

Small-scale lowland collectors also add value due to changes in place utility, because they often work for lowland traders by providing linkages between dispersed farmers and traders at a set rate (per bag). Thus, prices, transaction volumes, overall trade volumes, labor, returns to labor, and net incomes are all lower for small-scale lowland collectors than traders. Returns to labor for NK collectors and traders are twice as high as AC, probably due to lower returns for manure cake transactions in AC. Value is also added due to changes in time utility. Manure is transported to destinations at the time when it is needed for preparation of organic soil amendments by farmers. Traders add less value from changes in form utility, because dry manure is frequently removed from bags for sale by the cubic meter, as preferred by buyers. We found minimal evidence of possession utility, and a potential opportunity for credit in the region (below).

Daily wage laborers contributed to changes in place and time utility, were secondary beneficiaries of manure trade, and were seasonally hired by traders and collectors to gather, load and unload manure. We were not able to determine daily wages for hired laborers, because they were paid by the bag or cubic meter ($0.71/m^3). However, it is probably consistent with daily rates for agricultural labor in the study region (estimated at $2/d to $5/d).

4.2 Manure origin and supply

Most manure sales in NK and AC occur between February and August, which coincides with dry seasons (manure was easier to dry for transport) and high demand for organic amendments in regions purchasing manure. The period when farmers can dry manure does not usually compete with periods of demand for fertilization of lowland crops (e.g., rice), because farmers compost manure during the late dry season (August) and the rainy season (September to December) for application on the first rice crop (winter-spring), second rice crop (summer-fall), or forage crops. Stored composted manure from August or September to January is reportedly sufficient to fertilize crops at desired manure application rates for most farms. Minimal evidence of non-monetary manure trade exists in the regions surveyed, and probably occurs only on a local basis. Reported trade of composted manure (by two farmers) for rice straw and forage to feed cattle generates local nutrient cycles, which may be important to more evenly distributing nutrients in cattle manure on lowland agricultural lands.

Farmers in AC that do not have access to concrete slabs or paved roadside generally prepare manure for sale in cakes to prevent sandy soil contamination, although it is a less desirable product according to manure buyers, and may be accepted only by some buyers when mixed with broken-up manure. Furthermore, cake density is lower than broken-up manure, which combined with end user preference for broken-up manure, may explain the lower price of manure cakes. Farmers perceive that cakes without sand are more valuable than broken-up manure with some sand. Rubber companies are probably the primary consumers of manure cakes (often mixed with broken-up manure) for fertilization and commercial phân vi sinh production. Destination farmers do not knowingly purchase manure cakes, although they could be mixed with broken-up manure in some purchase transactions. Production of manure cakes despite lower prices and end user preference for broken-up manure suggests lack of information flow through the chain from end users to lowland cattle farmers. The use of tarps or plywood sheets to dry manure could permit AC farmers without access to concrete slabs or paved roadside to process manure in broken-up form.

Larger time investment (hours/m^3 dry manure) was expected for manure cake preparation (based on researcher observations), but not reflected in reported labor. Cakes are generally prepared by children and elderly, and this labor may have been valued lower than adult labor by the adult farmers that were surveyed.

Higher transaction volumes and transaction revenues in NK reflect larger manure bag sizes relative to AC, less frequent sales, and more semi-intensive cattle operations (less grazing time relative to AC). Annual household revenues were higher for NK despite lower household herd sizes than AC. Available manure per household is probably lower in AC, because of four more grazing hours/d relative to NK. However, returns to manure-related labor did not differ by commune.

Nhơn Khánh and AC communes could supply a maximum of 814 Mg DM/yr and 384 Mg DM/yr assuming 700 kg manure DM/animal per yr, manure collection only when animals are not away from the household grazing (i.e., in nearby vacant fields or communal lands), and 62% sales of available manure for households selling manure. Applying the same assumption to their respective districts, maximum potential manure supply from An Nhơn and Tuy An is 10,049 Mg/yr and 8,976 Mg/yr, respectively, potentially making an important contribution to highland and southeast coastal nutrient demands. Graphical representation of livestock density (Figure 5) suggests probable manure supply sources (provinces and districts). Due to manure bulk and transport costs, manure trade likely exists primarily among neighboring provinces (e.g., AC manure flows south to Bình Thuận while NK manure does not). It is possible that not all districts with high livestock concentrations are involved in manure trade.

4.3 Manure destinations

4.3.1 Soil characterization, constraints and opportunities for highland soils

Constraints to agricultural productivity on central highland soils in Vietnam include P fixation, Al toxicity, low cation exchange capacity, low plant available water capacity, compaction, and low K (Moody *et al.*, 2008). Cattle manure is an important source of organic matter, and can be effective when used together with inorganic fertilizer for maintenance of long-term soil productivity (Goyal *et al.*, 1999; Kaur *et al.*, 2005). The ability of cattle manure to increase soil organic matter, buffer soil pH, improve cation exchange capacity, supply P and K, and improve water holding capacity (Zingore *et al.*, 2008) directly addresses highland soil fertility challenges. Farmers and extension educators recognize the importance of cattle manure to soil fertility, and have incorporated cattle manure into yearly soil amendment strategies, especially for higher-value crops.

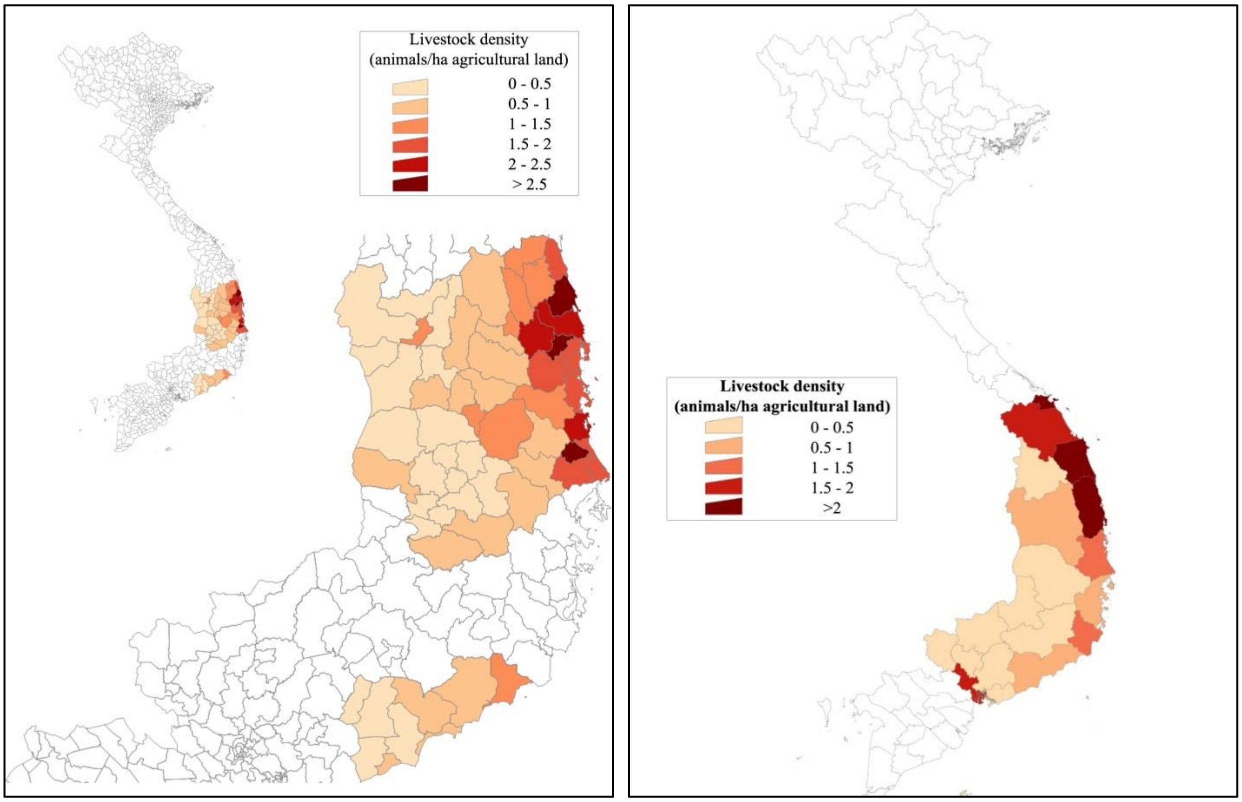

Figure 5 Livestock (cattle and water buffalo) density (animals/ha agricultural land) is indicated for districts in study Provinces (Bình Định, Phú Yên, Gia Lai, Đắk Lắk and Bình Thuận) in 2012 (left) (Binh Dinh Statistics Office, 2013; Binh Thuan Statistics Office, 2013; Dak Lak Statistics Office, 2013; Gia Lai Statistical Office, 2013; Phu Yen Statistics Office, 2013) and for Provinces in the south-central coast, Central Highlands, and southeast in 2013 (right) (Socialist Republic of Vietnam General Statistics Office, 2014).

4.3.2 Manure destinations and demand

The most common trader-reported destination for NK and AC manure was Chư Sê District in Gia Lai. The number of cattle and water buffalo in Chư Sê in 2012 was 19,554 (98% cattle) and there were 39,845 ha of agricultural land (including 2,131 ha pepper and 9,129 ha coffee), yielding a livestock (cattle and water buffalo) density of 0.491 animals/ha agricultural land

(Figure 5, Table 21). This density is much lower than lowland districts containing communes sampled in this study (2.54 animals/ha in An Nhơn and 2.13 animals/ha in Tuy An). Assuming 700 kg manure DM/animal per yr and 60% manure availability for agricultural use or sale in semi-intensive systems (Harsdorff, 2012), 8,213 Mg manure DM could be available from Chư Sê sources in 2012. Generously assuming all this manure could be allocated to pepper lands at farmer-defined rates (Table 14) of 4.43 Mg/ha per yr and coffee lands at 7.03 Mg/ha every two years, 41,529 Mg DM manure is required annually just to fertilize pepper and coffee lands. The 33,316 Mg DM/yr shortfall is actually much larger, because not all available manure is applied to higher value croplands. The deficit needs to be filled by manure purchased from other provinces and districts in south-central Vietnam (e.g., NK and AC communes) with higher animal densities and probable manure surpluses (Figure 5). Similar animal densities and manure shortfalls were calculated for other destinations in this study (Table 21). Mean district shortfall calculated for all districts in Gia Lai, Bình Thuận and Đắk Lắk is 22,612 Mg DM/yr, and most districts (24/42) experience shortfalls based on these calculations. Surpluses exist only in districts that produce little or no higher value crops.

Table 21 Livestock (cattle and water buffalo) density, manure demand for high value crop production, local potential supply, district manure shortfall, and the percentage of that shortfall that could be covered by Nhơn Khánh (NK) and An Chấn (AC) Communes in manure purchasing highland and southeast coastal districts.

Province	District	Animals/ha agricultural land	Coffee, pepper, dragon fruit demand (Mg DM/yr)	Local potential manure supply (Mg DM)	Shortfall (Mg DM/yr)	NK and AC supply (% of shortfall)
Bình Thuận	Hàm Thuận Bắc	0.838	69,041	17,321	51,719	2.3
Bình Thuận	Hàm Thuận Nam	0.475	118,014	8,966	109,048	1.1
Đắk Lắk	Buôn Hồ	0.290	60,884	2,922	57,962	2.1
Gia Lai	Chu Prông	0.299	56,970	9,355	47,615	2.5
Gia Lai	Chư Pưh	0.561	17,039	7,200	9,839	12.2
Gia Lai	Chư Sê	0.491	41,529	8,213	33,316	3.6
Gia Lai	Đăk Đoa	0.306	49,147	8,273	40,874	2.9

The overall yearly manure demand for dragon fruit production in Bình Thuận is calculated at 211,667 Mg DM, which is lower than demand for pepper and coffee production in Gia Lai (310,290 Mg DM) and Đắk Lắk (733,393 Mg DM). Manure traders consider sales to Bình Thuận as secondary manure trade income, because prices and overall demand are lower. Our investigations did not reveal direct manure value chain linkages between manure of Vietnamese origin and neighboring countries (i.e., Laos and Cambodia), although others have hypothesized that these linkages may exist (Birch *et al.*, 2014).

4.4 Actor labor and net incomes

Total yearly labor invested in manure by cattle farmers and traders followed a similar pattern to returns to labor (Table 19), suggesting the relative importance of manure to actor livelihoods (e.g., returns ($/hr) for large traders more than tripled returns for lowland cattle farmers). Net incomes (Table 20) again may indicate the relative importance of manure trade participation to livelihoods. Lowland farmers sell manure as an important supplemental form of income from cattle byproduct after accounting for household crop fertilization needs. Small-scale lowland collectors employ in manure trade on a seasonal basis, often in an agreement with traders. They are usually farmers, and manure collection income supplements farming income. The same is true for lowland compost traders, although these participants also work as oxcart drivers. In contrast, manure trade represents a primary source of income for lowland and highland traders. Truck owners combine seasonal manure trade with commercial trucking of other products. Some lowland traders are also farmers, but farming is not usually their primary income source. We were not able to evaluate the importance of manure-related income versus alternatives, but labor investment in manure suggests the perceived importance of the activity to actor livelihoods. Furthermore, seasonal fluctuation in manure-related labor suggests that manure trade is more important during pre-rainy season periods of peak manure trade activity.

4.5 Cattle and manure density hypothesis

Livestock (cattle and buffalo) density is higher per ha of agricultural land for coastal provinces in the study area and for coastal districts than for Central Highland Provinces and districts (Figure 5) (Binh Dinh Statistics Office, 2013; Binh Thuan Statistics Office, 2013; Dak Lak Statistics Office, 2013; Gia Lai Statistical Office, 2013; Phu Yen Statistics Office, 2013; Socialist Republic of Vietnam General Statistics Office, 2014). Thus, we hypothesize that livestock density plays a key role in the flow of manure from lowland south-central coastal regions to highland and southeast coastal destinations (Figure 5). Manure supply exceeds demand in many higher animal density lowland regions, while manure shortfalls exist in Central Highland and southeast coastal regions that cultivate higher value crops (e.g., pepper, coffee, dragon fruit and rubber) (Figure 6, Table 21).

Supporting this hypothesis, cattle numbers in Gia Lai districts receiving the highest flow of manure have continued to rise (Figure 7), although countrywide numbers and numbers for surrounding provinces have declined since 2007 from nearly 7 M in 2007 to just over 5 M in 2013 (Socialist Republic of Vietnam General Statistics Office, 2014). The continual rise in pepper and coffee production area in Gia Lai and Đắk Lắk since 2005 (not shown) and in dragon fruit production in Bình Thuận since 2008 has increased manure demand.

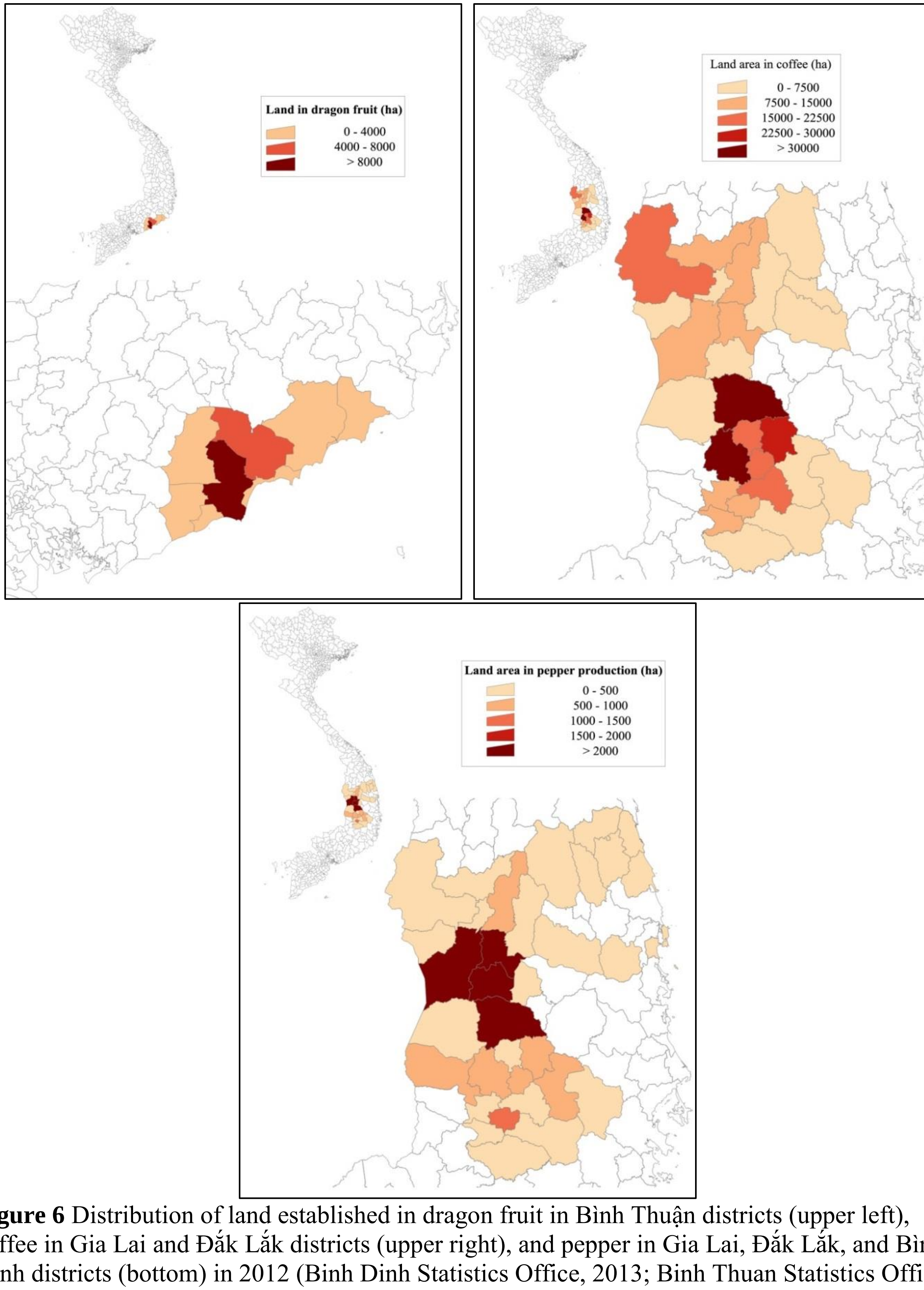

Figure 6 Distribution of land established in dragon fruit in Bình Thuận districts (upper left), coffee in Gia Lai and Đắk Lắk districts (upper right), and pepper in Gia Lai, Đắk Lắk, and Bình Định districts (bottom) in 2012 (Binh Dinh Statistics Office, 2013; Binh Thuan Statistics Office, 2013; Dak Lak Statistics Office, 2013; Gia Lai Statistical Office, 2013).

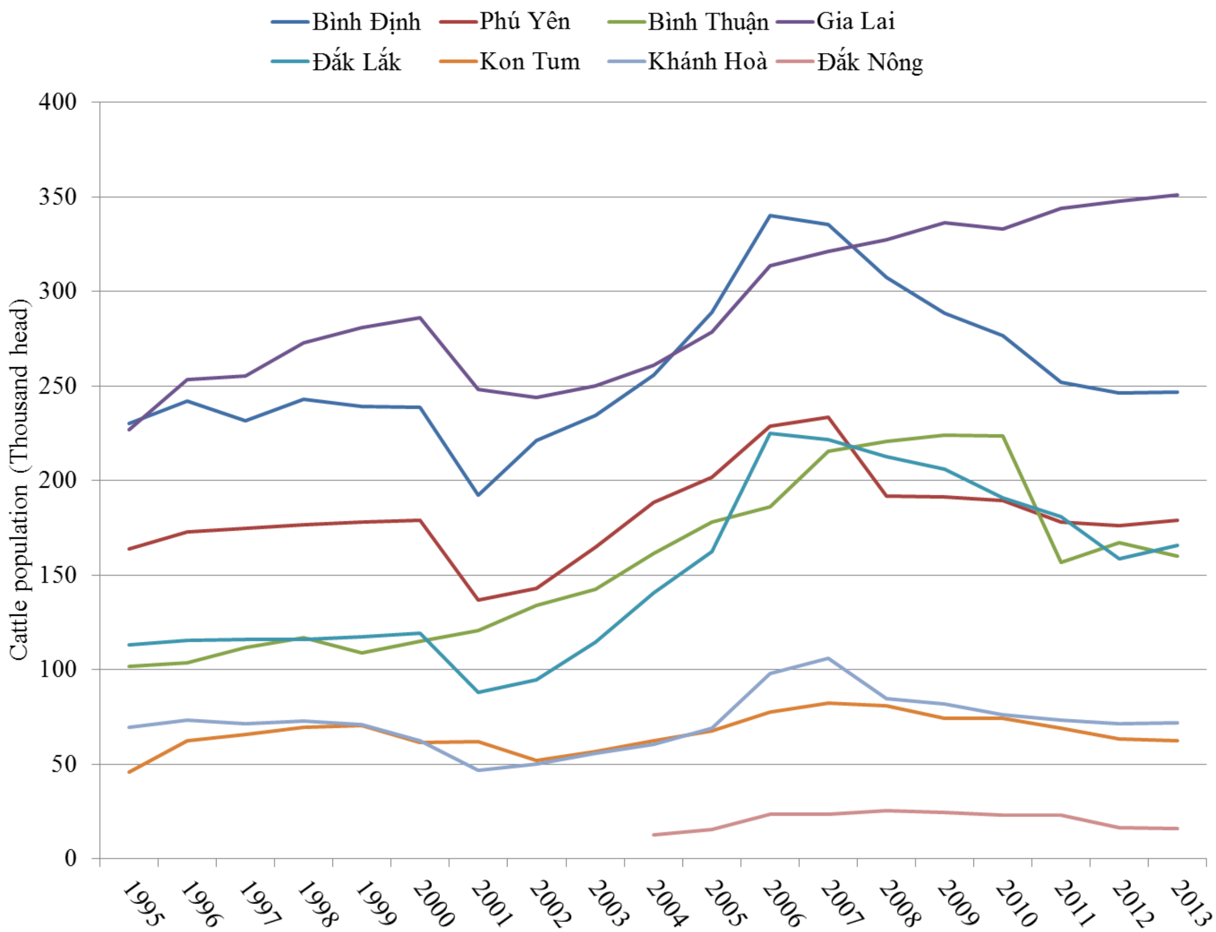

Figure 7 Cattle population in provinces in the south-central coast, southeast coast and Central Highlands from 1995 to 2013 (Socialist Republic of Vietnam General Statistics Office, 2014).

4.6 Manure price and fertilizer equivalence

Manure prices reported in this study are similar to others reported in the literature for Vietnam. Dan *et al.* (2004) reported 2003 manure prices in southeastern provinces near Hồ Chí Minh City at \$5.33 to \$8.00/m^3, versus \$16.27/m^3 in lowland farmer transactions in our study. Colson and Boutonnet (2006) reported composted hog manure prices at \$3.23/500 kg in north Vietnam, considerably lower than the \$9.00/500 kg composted manure reported here. The 2003 price of composted manure reported by Dan *et al.* (2004) was \$7.23/500 kg. They also observed the price of N in cattle manure at \$3.39/kg and N in compost prepared by farmers at \$0.98/kg, under the

$4.11/kg or $4.40/kg farmers reported in this study. McRoberts (Chapter 1) calculated the price of N in composted cattle manure in south-central coastal Vietnam at $2.86/kg.

Colson and Boutonnet (2006) observed in north Vietnam that N in cattle manure was five times more expensive than N in urea. Lekasi *et al.* (2001) reported that the organic fertilizer value of cattle manure was about five times more expensive than urea and triple superphosphate equivalents. In our study, N in manure sold by lowland farmers to traders was about 3.5 times the cost of N in urea, which destination farmers and rubber companies purchased from traders for 7.5 and 6.5 times the cost of N in urea, respectively. Most farmers in the Colson and Boutonnet (2006) study speculated that the high value of manure from a chemical equivalence standpoint reflects farmer perceptions about the role of manure in maintaining soil fertility and crop yields over time. We hypothesize that this is also the case in Vietnam, although it may not be a rational decision for buyers.

Colson and Boutonnet (2006) suggested that the high price of organic N in hog manure limited manure value chain development. We did not detect this constraint in south-central and southeast Vietnam. Finally, Colson and Boutonnet (2006) observed that cattle manure traders regard manure trade as a primary source of income and achieved incomes of about $2.90/day, much higher than the reported agricultural daily wage in 2006 ($1.33/d). This is consistent with our study, where trader incomes were higher than agricultural daily wages. Manure trade is quite lucrative, and is considered to be a primary source of income, exceeding $13,000/yr for lowland and highland traders.

Van *et al.* (2014) calculated the economic tradeoff between selling manure or applying it to forages in Bình Định Province. The income generated from sales of 20 Mg DM manure was approximately $952, while the value of increased forage yield from fertilization with 20 Mg manure DM/ha per yr was \$962. Calculations assumed 4.76 ¢/kg dry manure (slightly higher than 4.51 ¢/kg in AC to 4.65 ¢/kg in NK calculated in our study) and 23.8 ¢/kg forage DM. Using lowland manure value in our study, income generated from sales of 20 Mg DM manure was estimated at \$902 (transaction price) or \$930 (reported average yearly price). They suggested lower perceived risk of immediate returns from manure sales as a principal reason to sell (Van *et al.*, 2014). The resulting certainty equivalence is small, between \$10 and \$50/yr. McRoberts (Chapter 1) did not detect a forage yield response to composted cattle manure treatments if added without additional urea. Combined with low calculated certainty equivalence, this suggests low short-term opportunity cost to manure sales. The economics of tradeoffs in manure use versus sale are impacted by manure and forage value, labor, and manure and inorganic fertilizer application rates and prices, and merit further study to fully evaluate tradeoffs in manure allocation decisions.

4.7 Economic opportunities for origin and destination farmers

Destination highland farmers may benefit from buying manure when prices are lower, and by buying non-bagged manure (significantly lower cost than bagged manure). Highest prices were reported early in the year, and prices decreased at the onset of the highland rainy season in June or July. Cash availability often limits manure investment to post-harvest periods for coffee and pepper (early in the year). Similarly, lowland farmers could increase revenue from manure sales by storing manure from June or July onward to dry and sell early in the following year (dry season onset in January to March, when manure prices are highest). The benefits and pitfalls of these strategies require further investigation. Highland farmers may perceive elevated risk to invest during periods of constrained cash flow and uncertainty about future harvest returns. Credit may be required, but current availability and terms of credit systems in the region are not known. Cramb *et al.* (2004) reported available formal and informal credit in Đắk Lắk Province, but loan acquisition was often a difficult process with unattractive terms for farmers.

Investigation of crop response to manure application over time in lowland and highland regions can shed light on the decision to buy or sell manure versus other alternatives.

Better understanding of appropriate manure and fertilizer application rates could help mitigate over- (or under-) application, thus improving sustainability of agricultural production and possibly raising farmer net income. Excessive manure and fertilizer use has been documented (Hedlund *et al.*, 2003), and could elevate nutrient losses from runoff, volatilization, leaching, and denitrification. In regions where manure is over-applied, further development of manure trade systems can help redistribute nutrients from areas with high animal density relative to agricultural land (e.g., Ho Chi Minh City, south central coastal Vietnam; see Figure 5). Conversely, regions with negative nutrient balances could benefit by retaining more manure on farms. Sustainable, environmentally conscious application rates must be established from field experiments and modeling to support these nutrient management decisions.

4.8 Chain-level implications and possible value chain evolution

This study generated information about manure value chains in Vietnam that could inform future quantitative analysis of value chain dynamics via simulation modeling (Rich *et al.*, 2011). For example, changes in pepper, coffee, rubber and dragon fruit acreages can dramatically change manure demand. For example, pepper acreages increased by approximately 60% from 2008 to 2012 in Gia Lai and Đắk Lắk, and dragon fruit acreages nearly doubled in Bình Thuận during the same period (Binh Thuan Statistics Office, 2013; Dak Lak Statistics Office, 2013; Gia Lai Statistical Office, 2013). Lowland manure availability for fertilizer use and sale would be affected by government regulation of the currently informal manure trade sector (e.g., taxation), natural disasters or diseases impacting crops (demand shock) or animals (supply shock), and further transition from extensive grazing systems to semi-intensive or intensive cattle management systems (Ba *et al.*, 2013; Ba *et al.*, 2014). The impact of these shocks on manure value chains, crop-livestock systems, and actors at various levels should be better understood. Simulation of factors impacting manure trade system resiliency and weaknesses also merit consideration. Finally, assessment of the long-term economic and soil fertility impact of farmer participation in manure trade will indicate if trade is a favorable short- and long-term strategy under dynamic agricultural production conditions.

Detailed descriptive data from this study revealed several key questions for future investigation. First, the organic matter in manure is worth 20 to 50% more than alternative sources of organic matter. We hypothesize that this can be attributed to farmer perceptions about the ability of manure to maintain soil fertility over time relative to other organic matter alternatives, and farmer preference for manure even when prices are high. Future research should evaluate if this is a rational decision for manure buyers. Second, our study suggests that there is low opportunity cost for lowland farmers to sell manure relative to using it as an organic amendment on forages. Opportunity cost of manure use on other cash crops also merits consideration, although we hypothesize similar low short-term opportunity costs. Third, we did not evaluate the importance of participation in manure trade versus other income generating activities for chain participants. This is important step to understanding the relative importance of manure trade versus income generating alternatives, especially for lowland traders that rely on manure as a primary income source.

5. Acknowledgments

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APPENDIX 1: Crop fertilization rates reported by lowland cattle farmers

Table A1 Land area and fertilization rates by crop as reported by farmers in An Chấn (AC) and Nhơn Khánh (NK) communes. Land area means are calculated only from households that plant a given crop. Fertilization rate means are calculated only from households that use a given fertilizer. Composted manure dry matter rates were determined assuming 50% dry matter in applied compost.

Commune	Crop Name	Area (ha)			Compost (Mg DM/ha per yr)			Urea (kg/ha per yr)			NPK blends (kg/ha per yr)		
		n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
AC	Rice (winter-spring)	46	0.0868	0.0571	7	5.55	6.07	45	349	191	33	232	142
AC	Rice (summer-fall)	40	0.088	0.0429	12	6.72	4.79	39	349	165	31	217	147
AC	Rice (late year)	19	0.207	0.22	5	7.34	4.19	19	300	153	3	133	28.9
AC	Forage grass	47	0.0807	0.0630	29	7.90	9.67	47	805	1060	1	500	
AC	Maize	12	0.118	0.08	5	5.73	4.11	11	230	151	2	66.7	47.1
AC	Watermelon	10	0.09	0.0768	9	3.75	1.62	8	274	202	4	155	95.4
AC	Peanut	7	0.204	0.11	1	12.8		3	129	24.1			
AC	Cassava	7	0.168	0.152	1	0.8		3	128	75.2			
AC	Leafy vegetables	7	0.0531	0.0275	3	7.5	4.33	7	1250	964			
AC	Eggplant	3	0.0667	0.0289	3	15.3	8.08	2	320	113	1	800	
AC	Squash	3	0.127	0.108	3	5.71	3.74	2	140	84.9			
AC	Jicama	1	0.03		1	8.33		1	200				
AC	Gourd	1	0.04		1	6.25		1	250				
AC	Sesame	1	0.15										
NK	Rice (winter-spring)	48	0.223	0.129	34	6.71	5.41	45	177	87.6	48	212	96.7
NK	Rice (summer-fall)	50	0.225	0.125	9	2.9	2.41	48	193	97.6	49	226	105
NK	Forage grass	25	0.0638	0.057	7	2.02	1.72	23	705	604			
NK	Maize	36	0.112	0.0493	6	2.21	1.87	36	364	240	21	306	216
NK	Peanut	10	0.118	0.0442				1	500				
NK	Leafy vegetables	6	0.05	0.0158		4	2.83				5	736	355
NK	Watermelon	1	1		1	0.225		1	100		1	1100	
NK	Bonsai		150 trees			1 kg DM/tree per yr						0.48 kg/tree per yr	

Table A1, part 2

Commune	Crop Name	Diammonium Phosphate (kg/ha per yr)			Potassium blend (kg/ha per yr)			Phosphorus blend (kg/ha per yr)			Ammonium Sulphate (kg/ha per yr)			Lime (kg/ha per yr)		
		n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
AC	Rice (winter-spring)	12	126	78.3	30	111	94.9	12	121	72.9	4	106	30.3			
AC	Rice (summer-fall)	11	130	79.3	30	122	129	12	116	67.4	4	110	26.8			
AC	Rice (late year)				4	60	27.1	1	100		2	70	42.4			
AC	Forage grass				1	250					2	1300	707			
AC	Maize										3	261	67.4			
AC	Watermelon	8	160	62	4	97.9	45.3									
AC	Peanut				1	200		1	333		7	231	121			
AC	Cassava							1	500		7	398	206			
AC	Leafy vegetables															
AC	Eggplant	2	205	276				1	400							
AC	Squash	3	294	236												
AC	Jicama				1	333					1	333				
AC	Gourd															
AC	Sesame	1	20													
NK	Rice (winter-spring)	9	73.7	39.9	38	104	90.6	11	239	162	1	70				
NK	Rice (summer-fall)	10	90	34.1	40	105	88.6	13	251	164	1	70				
NK	Forage grass															
NK	Maize	3	300		10	291	321	16	373	157						
NK	Peanut				1	333		6	378	138				3	378	107
NK	Leafy vegetables							1	200							
NK	Watermelon	1	50		1	200		1	500							
NK	Bonsai															

Table A2 Land area and fertilization rates by crop field as reported by farmers in Bình Thuận and highland communes. Land area means are calculated only from fields that households plant to a given crop. Fertilization rate means are calculated only from households that use a given fertilizer on a given field. Composted manure dry matter rates were determined based on the assumption of 50% dry matter in compost applied.

Location	Crop Name	Land area (ha)			Compost (Mg DM/ha per yr)			Phân vi sinh (Mg/ha per yr)			Urea (kg/ha per yr)		
		n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
Bình Thuận	Dragon fruit	20	0.549	0.326	20	9.20	2.46				4	1430	593
Highland	Pepper	51	0.663	0.536	47	6.54	3.93	12	3.55	3.14	15	839	760
Highland	Coffee	38	1.03	0.788	24	5.58	1.91	6	2.02	1.28	20	1124	628
Bình Thuận	Rice	10	0.400	0.327							10	32.0	20.2
Highland	Rice	7	0.686	0.647							6	31.7	18.3
Highland	Maize	3	1.20	1.57							3	36.7	11.5
Highland	Rubber	1	0.600										
Highland	Cashew	2	2.00	0									
Highland	Sugarcane	1	0.200								1	200	

Table A2, Part 2

Location	Crop Name	NPK blends (kg/ha per yr)			Ammonium sulfate (kg/ha per yr)			Potassium blend (kg/ha per yr)			Phosphorus blend (kg/ha per yr)			NPK cao cấp (kg/ha per yr)		
		n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
Bình Thuận	Dragon fruit	20	4821	1590				2	2958	2551	7	2737	1016	6	5946	4034
Highland	Pepper	36	1982	1398	2	1550	1344	18	818	704	10	1931	1567			
Highland	Coffee	29	1692	747	7	965	636	23	753	870	4	1627	920			
Bình Thuận	Rice	8	22.5	2.7				8	14.8	9.6	2	10.0				
Highland	Rice	4	36.3	9.46	2	50.0	35.4	5	26.0	23.0	1	35.0				
Highland	Maize	3	36.7	12.6				2	35.0	21.2						
Highland	Rubber	1	417													
Highland	Cashew				2	188	88.4									
Highland	Sugarcane										1	300				

APPENDIX 2: Feed ingredients and rates reported by lowland farmers for cattle

Table A3 Ingredients fed to cattle as reported by farmers in An Chấn and Nhơn Khánh communes, and the mean and SD of the amount fed. Not all respondents were able to report daily quantities fed. Total farmers interviewed in AC and NK were 50 and 51, respectively.

	AC				NK			
Ingredient (as fed)	Frequency (# of respondents)	Amount fed (kg/animal per d)			Frequency (# of respondents)	Amount fed (kg/animal per d)		
		n	Mean	SD		n	Mean	SD
Concentrate	-	1	1		-			
Dried cassava	4	4	0.258	0.162	-			
Fresh grass	44	44	16.3	12.6	21	18	14.7	7.85
Maize	1	1	16.7		6	5	0.303	0.123
Rice [a]	28	27	0.428	0.932	25	23	0.746	2.02
Rice bran	19	19	0.375	0.324	13	13	0.474	0.326
Rice straw	47	47	3.85	4.53	12	9	5.81	2.55
Salt	1	1	0.0450		-			
Water spinach or leafy vegetables	31	28	2.27	1.21	21	20	3.80	2.29

[a] Rice was ground up and fed in water.

APPENDIX 3: Questionnaires for manure value chain analysis

Questionnaire for lowland farmers that are cattle owners

Code #:____________
Interviewer Name ____
Date ____
Household Identifier ____
GPS Coordinates ____

1. Do you own cattle, rent cattle, or keep cattle on your property? Yes ___ No ___
If no, discontinue questionnaire.

2. If yes, do you sell cattle manure or have you ever sold cattle manure? Yes ___ No ___
If no and do not purchase manure, only answer questions 5-9, 11-12, 19, and 25

3. Is this manure pure cattle manure? Yes ____ No _____
If no, please indicate percentage of different sources or additives:
Cattle Manure _________% Chicken Manure _________%
Hog Manure _________% Rice Straw _________%
Soil _________% Other (specify)_____________________, ______%

4. Do you purchase manure? Yes ____ No ____
If yes, do you purchase manure for use on your farm or for resale? On farm _____ Resale ____
If for resale, also complete manure collector questionnaire.
If purchase but don't sell, answer questions 5-9, 11-12, and 19-25.

5. How many cattle do you own, rent, or house on your property during the past year? ________

Category	Number	Management system (intensive, extensive, semi-intensive)
Water buffalo		
Cows		
Calves (<1yr)		
Bulls		
Other:		

5.1 For semi intensive and extensive management, please indicate the approximate amount of time each day that your animals spend grazing during different seasons and distance from household

Season or Time Period	Average Daily grazing time (hours/day)	Average distance from household	Manure collected when grazing (Y/N), If Y, give % collected

5.2 Please list the most important components of cattle diets in descending importance.

Forage, Feedstuff or Byproduct Name	Notes

6. Do you own or rent land? Yes ____ No ____
If yes, how much? Owned: ____________________ Rented: _____________________

7. What percentage of the manure on your farm is sold? __________________%

8a. Why do you choose to sell (or not sell) manure?

____
____
____
____
____
____
____
____

8b. What other alternatives do you have for manure use?

9a. What are your greatest challenges related to manure management?

9b. What are your greatest risks related to manure management?

10. Is there anything different about the manure you keep versus sell? Are they valued the same or differently?

11. Please describe specifically how you gather, store, process or prepare the manure for sale and sell it, including the time invested in each task and the amount of time each step requires.

Dried Patty Manure Preparation and Marketing or Sales Step	Manure Quantity	Time required in labor	Time required to complete each step
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			

____

____

____

____

Dried Broken-Up Manure Preparation and Marketing or Sales Step	Manure Quantity	Time required in labor	Time required to complete each step
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			

____

____

____

____

Composted Manure Preparation and Marketing or Sales Step	Manure Quantity	Time required in labor	Time required to complete each step
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			

____

____

12. Would you like to provide additional information about the manure preparation process?

____

____

____

____

____

13. Please describe recent manure sales transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the buyer or destination of the product for each (if known)

Transaction 1					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other)	Amount (indicate units)	Price (VND)	Buyer, Destination and buyer class
Manure Transport method and distance to buyer: Notes about Transaction:					

Transaction 2					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Buyer, Destination and buyer class
Manure Transport method and distance to buyer: Notes about Transaction:					

Transaction 3					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Buyer, Destination and buyer class
Manure Transport method and distance to buyer: Notes about Transaction:					

Transaction 4					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Buyer, Destination and buyer class
Manure Transport method and distance to buyer: Notes about Transaction:					

Transaction 5					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Buyer, Destination and buyer class
Manure Transport method and distance to buyer: Notes about Transaction:					

14. What are your ideas to improve the transaction process?

___

___

___

___

___

15. Have you ever wanted to sell manure but did not? If so, please describe why you were unable to sell it.

____
____
____
____
____
____

16. What is the quantity of manure sold annually and the average value per unit sold (define units)?

Quantity sold annually (or broken down by season):

____
____
____

Average Price per unit: ____________ Maximum: ______________ Minimum: ____________

____
____
____

17a. If you were unable to sell manure, what would you do with it?

____
____
____
____

17b. How would this affect other aspects of nutrient management on your farm?

____
____
____
____
____
____

18. Is there anything else you would like to tell us about the production and marketing system for cattle manure?

____
____
____
____
____
____
____

19. Other comments:

____
____
____
____
____
____
____

20. Manure purchased for household use.
Please describe recent manure purchase transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the seller for each (if known)

Transaction 1					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other)	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Manure Transport method and distance from seller: Notes about Transaction:					

Transaction 2					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Manure Transport method and distance from seller: Notes about Transaction:					

Transaction 3					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Manure Transport method and distance from seller: Notes about Transaction:					

Transaction 4					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Manure Transport method and distance from seller: Notes about Transaction:					

Transaction 5					
Transaction Date	Payment Date	Type of Manure	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Manure Transport method and distance from seller: Notes about Transaction:					

21. What do you do with the manure you purchase? How is it used or what crops or land is it applied to? Does the destination of use depend on distance from the household?

____
____
____
____
____
____

22. How much manure do you purchase annually and what is the price per unit (indicate units)?

Amount purchased annually or seasonally: ______________________________________

Average price: ____________ Maximum: ___________ Minimum: ________________

____

23. If you were unable to purchase manure, how would it impact nutrient management on your farm?

____
____
____
____

24. Is there anything else you would like to add about the manure you purchase for use on farm?

____
____
____
____
____
____

25. Please describe all nutrient applications for all crops planted during the past year.

Fertilizer name and composition	**Crop and amount of each applied for each crop, date**		
	1.____________ Area: ________ Date: ________ Distance: ________	**2. ____________ Area: ________ Date: ________ Distance: ________**	**3. ____________ Area: ________ Date: ________ Distance: ________**
Cattle Manure			
Urea (46:0:0)			
Fertilizer name and composition	**Crop, total amount of each applied for each crop, date of establishment**		
	4.____________ Area: ________ Date: ________ Distance: ________	**5. ____________ Area: ________ Date: ________ Distance: ________**	**6. ____________ Area: ________ Date: ________ Distance: ________**
Cattle Manure			
Urea (46:0:0)			

Fertilizer name and composition	**Crop, total amount of each applied for each crop, date of establishment**		
	7.____________ **Area: ________** **Date: ________** **Distance: ________**	**8. ____________** **Area: ________** **Date: ________** **Distance: ________**	**9. ____________** **Area: ________** **Date: ________** **Distance: ________**
Cattle Manure			
Urea (46:0:0)			

Questionnaire for local manure traders and collectors

Interviewer Name ____

Date ____

Identifier ____

GPS Coordinates ____

1. What is your primary occupation? ________________________________

2a. Do you purchase manure and/or act as a regional manure collector? Yes _____ No _____

If no, discontinue questionnaire.

If yes, do you purchase manure for:

Use on your farm _______

To sell to other farmers _______

To sell to transportation intermediaries to highlands _______

To intermediaries (destination unknown) _______

Other (please specify) _________________________________

2b. What types of manure do you collect? Please indicate the animal type and the estimated proportion of your collection business

Cattle ____

Hog ____

Chicken____

Other ____

2c. Please indicate the proportion of your cattle manure collection business that is:

Ground up: _____________________

Round patty: ____________________

Compost: _______________________

2d. Are the different manures mixed or kept separate for sale? Please describe.

____

____

____

____

____

____

3a. Please describe the geographical region from which you collect manure.

____
____
____
____
____
____

3b. Where are the collection points for the manure that you collect?

____
____
____
____
____
____

4. Do you have a contract with farmers to buy manure? Yes _____ No _____
If yes, is the contract: Written ____ Oral ____ Other ____

Do you have a contract with transporters or buyers to sell manure? Yes ____ No ____
If yes, is the contract: Written ____ Oral ____ Other ____

Please describe the nature of the contract or contracts (e.g., annual or monthly quota? Fixed prices?).

____
____
____
____

5.1 Do you own a truck for hauling manure? Yes ____ No ____
Do you own an ox cart for hauling manure? Yes ____ No _____

6. How many other manure collectors are in the region?

____
____
____

7. Is there seasonal variation in the amount of time you spend on manure collection?
Yes __________ No ___________

If yes, how many hours do you allocate in an average week to manure collection during different seasons?

Season	Hours per week, day or other unit of time

8. Please describe the seasonal labor differences

____
____
____
____
____
____

9. Please describe the typical steps that are necessary to find this manure, collect it, store it, prepare it, market it, and sell it. How much of your time is required for each step on average?

Manure collection, storage, preparation, transport, marketing, and sales steps	Time required in labor per event – also indicate manure amount	Time required to complete each step
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		

____
____
____
____
____
____

10. How is the manure stored between purchase and sale?
Covered _________, Is so, with what? __________________________
Bagged _________, If so, do farmers bag it or do you bag it? ________ Material? ________
Other (please specify) ____
____
____
____
____
____
____

If bagged or stored in a container, how large is the bag or container? ______________________

11. Describe anything you have done to the manure between purchase and sale (e.g., bagging, drying, adding straw, composting) and why it is done.
____
____
____
____
____

12. Would you like to add additional information about the labor involved in the process?
____
____
____
____
____
____
____

13. Please describe recent manure purchase transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the seller and location of the product for each (if known)

Transaction 1 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transportation method from seller, distance, and cost: Notes:					
Transaction 2 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transportation method from seller, distance, and cost: Notes:					

Transaction 3 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transportation method from seller, distance, and cost: Notes:					

Transaction 4 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transportation method from seller, distance, and cost: Notes:					

Transaction 5 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transportation method from seller, distance, and cost: Notes:					

14. Please describe recent manure sales transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the buyer or destination of the product for each (if known)

Transaction 1 - sale					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyer, Destination and Buyer Class
Transportation method to buyer, distance, and cost: Notes:					

Transaction 2 - sale					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyer, Destination and Buyer Class
Transportation method to buyer, distance, and cost: Notes:					

Transaction 3 - sale					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyer, Destination and Buyer Class
Transportation method to buyer, distance, and cost: Notes:					

Transaction 4 - sale					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyer, Destination and Buyer Class
Transportation method to buyer, distance, and cost: Notes:					

Transaction 5 - sale					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyer, Destination and Buyer Class
Transportation method to buyer, distance, and cost: Notes:					

15a. What is the quantity of manure purchased annually (or monthly or seasonally) and the average value per unit purchased (define units)?

Annual, Monthly or Seasonal Quantity:

____

____

____

____

____

Average Price per Unit: _________________ Minimum: ___________ Maximum: ___________

15b. What is the quantity of manure sold annually and the average value per unit sold (define units)?

Annual, Monthly or Seasonal Quantity:

____

____

____

____

____

Average Price per Unit: _________________ Minimum: ___________ Maximum: ___________

16. Do you have ideas to improve the transaction processes?   Yes ____ No ____
Please explain.

____
____
____
____
____
____

17a. Is there a difference between purchased and sold amounts of manure? If so, why do you think this difference occurs?

____
____
____
____
____

17b. What percentage change do you think occurs? _______________%

18. Do you evaluate manure quality during the collection process? If so, please explain how you determine the quality of the manure and if it affects the price.

____
____
____
____
____

19. Please indicate the percentage of your total manure sales that go to different principle destinations or types of buyers.

Destination or Buyer Type	% of Total Manure Sales

20a. What are your greatest challenges related to manure collection and sale?

20b. What are your greatest risks related to manure collection and sale?

21. What are your greatest opportunities related to manure collection and sale?

22. Have there been times when you wanted to buy and sell manure, but could not? Please Explain

23. What would you do differently in your business if you were unable to buy and sell manure?

24. Is there anything else you would like to tell us about the collection, marketing and transport system for cattle manure?

25. Other comments:

Questionnaire for highland manure traders

Interviewer Name ____

Date ____

Household Identifier ____

GPS Coordinates ____

1a. Do you purchase cattle manure to resell? Yes _____ No _____

If no, discontinue questionnaire.

1b. What types of manure do you sell and what proportion of your manure sales comes from each? Please indicate if any manures are mixed.

Cattle ____

Hog ____

Chicken ____

Other ____

2. Do other businesses also sell manure in your commune? District? How many?

____

____

____

3. Do you have a contract with sellers to buy manure? Yes _____ No _____

If yes, is the contract: Written ____ Oral ____ Other ____

Please describe the nature of the contract.

____

____

____

____

4a. Do you have a contract with transporters to transport manure? Yes ____ No ____

If yes, is the contract: Written ____ Oral ____ Other ____

Please describe the nature of the contract.

____

____

____

____

4b. Do you have a contract with buyers? Yes ____ No ____
If yes, is the contract: Written ____ Oral ____ Other ____
Please describe the nature of the contract.

5. Do you evaluate manure quality to determine prices? Yes ____ No ____
If yes, please describe how you evaluate manure quality and how it affects manure pricing.

6a. Do you have a truck? Yes _____ No _____
If so, do you use it for manure transportation events? Yes ____ No _____
How do you use it for manure transportation? (from lowland farms or other sellers to your business? From your business to highland buyers or transporters?

6b. Does manure distribution include delivery? Yes _______ No _______
Please describe.

7. How is manure stored prior to sale?
Outside in a covered pile ______
Outside in an uncovered pile ______
Outside in bags ______
Inside in a pile ______
Inside in bags ______
Other (please specify) ____

8. What percentage of the manure you deal is sold to:
Farmers __________________% Farmer type _____________________
Transporters ______________%
Companies _______________%
Other ________________% Indicate category. ___________________________________

9. Please describe the process of manure acquisition, storage, processing, and sale.
____
____
____
____
____
____
____
____
____
____

10a. Please describe recent manure purchase transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the seller and location of the product (if known)

Transaction 1 – purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transport method, distance, and cost: Notes on transaction:					

Transaction 2 – purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transport method, distance, and cost: Notes on transaction:					

Transaction 3 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transport method, distance, and cost: Notes on transaction:					

Transaction 4 – purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transport method, distance, and cost: Notes on transaction:					

Transaction 5 - purchase					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Seller and Location
Transport method, distance, and cost: Notes on transaction:					

10b. Please describe recent manure transport transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the transporter and location of origin of the product.

Transaction 1 – transport					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Transporter and distance
Notes:					
Transaction 2 – transport					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Transporter and distance
Notes:					

Transaction 3 – transport					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Transporter and distance
Notes:					

Transaction 4 – transport					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Transporter and distance
Notes:					

Transaction 5 – transport					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) & quality ranking (low, medium, high)	Amount (indicate units)	Price (VND)	Transporter and distance
Notes:					

10c. Please describe recent manure sales transactions, indicating the transaction dates, payment date, manure type, the amount, the price, and the buyer and destination of the product (if known).

Sales transactions 1					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyers and Location
Transaction notes:					

Sales transactions 2					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyers and Location
Transaction notes:					

Sales Transactions 3					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyers and Location
Transaction Notes:					

Sales Transactions 4					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyers and Location
Transaction Notes:					

Sales Transactions 5					
Transaction Date	Payment Date	Type of Manure & quality ranking	Amount (indicate units)	Price (VND)	Buyers and Location
Transaction Notes:					

11a. Does the price of manure you purchase and sell vary seasonally? ______________________

Please explain how it varies.

____
____
____
____

11b. Does the volume of manure you purchase and sell vary seasonally? ___________________
Please explain how it varies.

____
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____
____

12a. How many manure sales do you make each year, month or season? What is the average amount sold and the average price for each sale?

Manure sales per year (broken down by season):

____

Average amount sold per sale event: ____

Average Price: ______________ Maximum: _____________ Minimum: ________________

Notes:____

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12b. How many manure purchases do you make each year? What is the average amount purchased and the average purchase price for each purchase event?

Average purchase amount: ____

Average Price: ______________ Maximum: _____________ Minimum: ________________

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12c. If you make the transport arrangements and pay for manure transport, how many transport events do you arrange each year, what is the average amount of manure transported and the average amount paid for each transport event?

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12d. What is your approximate yearly (or seasonal) revenue from manure sales?

____

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13. What are the primary challenges to the manure transactions? (List most important first)

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14. Do you have ideas to improve the transaction processes? ______________________
Please explain.

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15. If you were not able to buy and sell manure, how would it impact your business?

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16a. What are your greatest challenges related to manure purchase and sale?

____

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16b. What are your greatest risks related to manure purchase and sale?

____

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____

17. What are your greatest benefits or opportunities related to manure purchase and sale?

18. Is there anything else you would like to tell us about the marketing and transport system for cattle manure?

19. Other comments:

Questionnaire for rubber companies that buy cattle manure

Interviewer Name ____________________
Date ____________________
Household Identifier ____________________
GPS Coordinates ____________________

1. Do you purchase cattle manure? Yes ____ No _____
If no, discontinue questionnaire.

2. Please explain why you purchase manure and how you use it.

3. Please describe a typical manure purchase transaction from the point of first contact with sellers to the point of receiving it on your farm?

4. Please describe recent manure purchase transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the seller for each (if known)

Transaction 1					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) and quality (1=low, 2=medium, 3=high)	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 2					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 3					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 4					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 5					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

5. What is the quantity of manure purchased annually and the average value per unit purchased (define units)?

Quantity purchased annually (or broken down by season):

____
____
____
____
____

Average Price per unit: ____________ Maximum: ______________ Minimum: ____________

____
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____
____
____

6a. What are the primary challenges to the transactions?

____
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6b. Do you have ideas to improve the transaction process?

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____
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7a. How do you judge manure quality (if at all)?
Please describe:

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7b. Do you pay a different price for high quality manure? Yes _____ No _____
Please describe: ____

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____

____

8. Do you have other sources of cattle or animal manure other than purchased manure?
Yes _____ No _____
If yes, what are the sources?

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9. What are your greatest challenges related to nutrient management?

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10. What are your greatest opportunities related to nutrient management?

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11. Is there anything that could be done to the manure you purchase that would make it more valuable for you? e.g., add or remove rice straw or other material, bag it, compost it, dry it, bring it fresh, etc.)

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12. If you could not purchase cattle manure, how would it affect the nutrient management decisions on your rubber company? What would you do differently or what substitute would you seek?

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13. How much land does your company have in rubber production? ________________

15. Please describe yearly rubber nutrient applications for your company with amounts and units.

Fertilizer name and composition	**Application period and amount of each applied**		
	1.___________ Area: ________ Crop Year:_________	**2. ___________ Area: ________ Crop Year:_________**	**3. ___________ Area: ________ Crop Year:_________**
Cattle Manure			
Urea (46:0:0)			

Fertilizer name and composition	Application period and amount of each applied		
	4.____________ Area: _________ Crop Year:__________	**5. ____________ Area: _________ Crop Year:__________**	**6. ____________ Area: _________ Crop Year:__________**
Cattle Manure			
Urea (46:0:0)			

16. Is there anything else you would like to tell us about the market, transport, and pricing system for cattle manure?

____

17. Other comments:

____

Questionnaire for coffee, pepper, or dragon fruit farmers that buy cattle manure

Interviewer Name ______________________________
Date ______________________________
Household Identifier ______________________________
GPS Coordinates ______________________________

1. Do you purchase cattle manure? Yes ____ No _____
If no, discontinue questionnaire.

2. Please explain why you purchase manure and how you use it.

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3. Please describe a typical manure purchase transaction from the point of first contact with sellers to the point of receiving it on your farm?

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4. Please describe recent manure purchase transactions, indicating the transaction date, payment date, manure type, the amount, the price, and the seller for each (if known)

Transaction 1					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) and quality (1=low, 2=medium, 3=high)	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 2					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 3					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) and quality (1=low, 2=medium, 3=high)	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 4					
Transaction Date	Payment Date	Type of Manure and quality	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

Transaction 5					
Transaction Date	Payment Date	Type of Manure (Fresh, composted, dried, dried and bagged, other) and quality (1=low, 2=medium, 3=high)	Amount (indicate units)	Price (VND)	Seller, Location, and Delivery method
Notes: Distance:					

5. What is the quantity of manure purchased annually and the average value per unit purchased (define units)?

Quantity purchased annually (or broken down by season):

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Average Price per unit: _____________ Maximum: _______________ Minimum: _____________

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6a. What are the primary challenges to the transactions?

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6b. Do you have ideas to improve the transaction process?

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7a. How do you judge manure quality (if at all)?
Please describe:

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7b. Do you pay a different price for high quality manure? Yes _____ No _____
Please describe: ____
____

8. Do you have other sources of cattle or animal manure other than purchased manure?
Yes _____ No _____
If yes, what are the sources?

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9. What are your greatest challenges related to nutrient management?

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10. What are your greatest opportunities related to nutrient management?

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11. Is there anything that could be done to the manure you purchase that would make it more valuable for you? e.g., add or remove rice straw or other material, bag it, compost it, dry it, bring it fresh, etc.)

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12. If you could not purchase cattle manure, how would it affect the nutrient management decisions on your farm? What would you do differently or what substitute would you seek?

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13. Do you own land? _________ rent land? ________
If yes, how much? Owned ____________ Rented ____________

14. Please describe all nutrient applications in 3 previous cropping seasons with amounts and units.

Fertilizer name and composition	**Crop and amount of each applied for each crop, crop year or date**		
	1.____________ **Area: ________** **Crop Year:_________**	**2. ____________** **Area: ________** **Crop Year:_________**	**3. ____________** **Area: ________** **Crop Year:_________**
Cattle Manure			
Urea (46:0:0)			

Fertilizer name and composition	**Crop, total amount of each applied for each crop, crop year or date**		
	4.___________ Area: ________ Crop Year:_________	**5. ___________ Area: ________ Crop Year:_________**	**6. ___________ Area: ________ Crop Year:_________**
Cattle Manure			
Urea (46:0:0)			
Fertilizer name and composition	**Crop, total amount of each applied for each crop, crop year or date**		
	7.___________ Area: ________ Crop Year:_________	**8. ___________ Area: ________ Crop Year:_________**	**9. ___________ Area: ________ Crop Year:_________**
Cattle Manure			
Urea (46:0:0)			

15. Is there anything else you would like to tell us about the market, transport, and pricing system for cattle manure?

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16. Other comments:

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CHAPTER 4: APPLICATION OF LOCAL BINARY PATTERNS IN DIGITAL IMAGES TO ESTIMATE BOTANICAL COMPOSITION IN MIXED ALFALFA – GRASS FIELDS

Abstract

Botanical composition in mixed stands of alfalfa and grass is a critical parameter in equations estimating harvest fiber concentration for dairy rations. Composition is difficult to estimate by visual observation. Digital image analysis in mixed stands could reduce botanical composition uncertainty and improve spring harvest management decisions. We sampled mixed stands (*n*=168) in farmers' fields in Tompkins County, New York in May 2011. A digital image was taken of standing samples at 5-Megapixels resolution using a Canon PowerShot A3100IS, and alfalfa and grass height relationships were recorded. After clipping representative samples at 10-cm above ground level, samples were manually separated into alfalfa (*Medicago sativa* L.) and timothy grass (*Phleum pratense* L.), and dried to calculate fractions on a dry matter basis. Uniform rotation invariant local binary patterns (LBP) were extracted from whole images and 64 x 64 pixel tiles, and were used to develop regression equations estimating grass fraction. Tiles were manually classified as alfalfa (0), grass (1) or unclassifiable. An iterative process selected most accurate local binary pattern operator settings. Grass fraction was estimated in three regression model development approaches: (1) using average tile LBP histogram bins from whole images and botanical height relationships, (2) developing a binary tile classification model from tile LBP histogram bins, and using tile model-predicted grass probability averaged for tiles in whole images (grass coverage estimate) and botanical height relationships as inputs in whole image models, and (3) using LBP histogram bins extracted directly from whole images (1024 by 1024 pixel square) and height relationships. Predictive accuracy in whole image models using tile LBP histogram averages was highest for models generated from LBP tile histogram bin means (R^2_{pred} up to 0.847), followed closely by combined tile models and whole image models

(R^2_{pred} up to 0.807), with pairwise correlations between tile model-generated grass coverage estimates and sample grass fraction up to 0.895. LBPs are effective in differentiating alfalfa and grass under field conditions, because the method is robust to changes in color and illumination. Furthermore, key LBP histogram bins (e.g., symmetric edges) strongly differentiate alfalfa and grass in tiles. The LBP method is promising based on this study, but further evaluation under diverse field conditions, including different cameras and grass species, is necessary to assess usefulness.

1. Introduction

Timing of spring forage harvests in the northeast U.S. is critical to ensure high quality forage for dairy cattle production throughout the growing season. Spring forage harvest timing can be predicted based on neutral detergent fiber (NDF) concentration (Parsons *et al.*, 2006b), and target NDF depends on the class of livestock being fed when forages are the principal source of fiber in rations. Target NDF at harvest for dairy cattle is approximately 500 g/kg dry matter (DM) for pure grass stands for silage and 400 g/kg DM for alfalfa (Cherney *et al.*, 2006). Other forage quality parameters, such as protein and fiber digestibility, are important for ration balancing, but they are not as useful for harvest date targets.

Forage sampling of farmers' fields in New York has produced simple equations for the prediction of nutritive value and harvest timing for pure stands of alfalfa (*Medicago sativa* L.) , grass (e.g., *Phleum pratense* L., *Phalaris arundinacea* L., *Dactylis glomerata* L., and *Festuca arundinacea* Schreb.), and mixed stands of alfalfa and grass (Parsons *et al.*, 2006a; Parsons *et al.*, 2009; Parsons *et al.*, 2012). Forage stands can also be tested for dry matter loss and changes in NDF at variable stubble height, another important management factor (Parsons *et al.*, 2009; Parsons *et al.*, 2012). These equations have proven useful over a range of conditions and years.

Alfalfa is sown with a perennial grass companion on over 80% of the alfalfa acreage in New York (Cherney *et al.*, 2006), and accurate mixed stand equations are important for effective harvest management. Required inputs for mixed stand equations include alfalfa maximum height, stand composition (grass fraction in the stand), and targeted harvest NDF concentration (Parsons *et al.*, 2006a). Botanical or stand composition is a critical parameter in the equation, and is difficult to accurately predict by visual observation (McRoberts *et al.*, 2012a). Difficulty in estimating stand composition was reported by extension educators at the Cornell Field Crop Extension Educators' Retreat in April 2011 as the principle problem limiting the utility of mixed-stand equations for farmers in the northeast U.S. Misestimating composition by just 20% can result in late harvests by five or more days, potentially leading to NDF at harvest > 50 g/kg past target levels. This represents critical potential nutritive and economic losses for dairy farms. Reducing uncertainty in the stand composition estimate could improve the quality and timing of spring forage harvests.

Manual and semi-automated approaches have been attempted to estimate botanical composition in legume-grass stands. Visual estimation methods have been used historically as the principal method, including a visual guide for mixed stands of alfalfa and grass for agricultural extension purposes in New York (Parsons, unpublished). Rayburn and Green (2014) developed a visual reference guide for mixed stands of clover and grass to help calibrate the eye for human field estimation. Rayburn (2014) tested a manual point count method by iteratively superimposing a randomly placed virtual point count grid on mixed stand images, counting the number of points touching grass, legumes, forbs, bare ground, and dark shadows, and quantifying the points. Point counts were then regressed on actual botanical composition. The method was strong when using at least100 points per image (R^2 ranging from 0.45 to 0.98). However, it is time consuming and would require manual image processing by users as well as equation calibration with different species combinations, sampling seasons, and cameras. Height relationships of species sampled were not used in their method development.

In a pot experiment, Himstedt *et al.* (2009) discovered high correlations between legume coverage and actual legume DM fraction in mixed stands (R^2=0.89 across three legume species for two sward ages; R^2=0.96 for alfalfa). Actual legume coverage was calculated by manually circling areas covered by legumes and dividing by total area. They also estimated coverage by processing grayscale images using morphological operators, including a multi-step erosion process, followed by a dilation process with the same number of steps. Erosion effectively removed small objects such as grass leaves, while dilation blew the remaining objects (inner portion of alfalfa leaves) back up to their approximate original size. Grayscale thresholding was used to separate legume leaves from everything else in the image, and to estimate coverage as *legume leaves/total area*. The relationship between actual coverage and estimated coverage was strong for samples with higher percent coverage (R^2=0.88 overall, R^2=0.84 for alfalfa). However, sample size was small (64 images) and lighting and growth conditions were controlled.

Himstedt *et al.* (2010) furthered equation development using logit-transformed legume coverage in statistical model development, and selected a multivariate model predicting legume DM contribution with effects including logit-transformed legume coverage, total biomass in the sample, and their interaction. Equation testing on field samples yielded a strong relationship with legume DM contribution (R^2=0.98) for clover – grass mixes. Practicality of such an equation for field use is questionable without further investment in technology such as field spectroscopy given the need for the total DM biomass variable. Single cameras were used in all tests. The technique would require further testing under variable field conditions to evaluate potential field use.

In a field study, Post *et al.* (2007) related plant canopy spectral reflectance (wavelengths 680 nm and 705 nm in the second derivative spectra) with alfalfa fraction in a mixed stand (R^2= 0.6 to 0.7, n=95). The approach is promising for further investigation, and potentially for post-calibration field use (Post *et al.*, 2007). Others have implemented variations on canopy spectral reflectance to predict stand composition with promising results (Kawamura *et al.*, 2011). However, spectral technology may not be accessible for end users.

More sophisticated image processing methods such as artificial intelligence (Aitkenhead *et al.*, 2003) and texture classification (Sabeenian and Palanisamy, 2010) have been tested to discriminate between vegetation types (e.g., crops and weeds). Methods that permit crop – weed discrimination in real time, combined with robotic herbicide application and cultivation systems play an important role in precision agriculture. Local binary patterns (LBP), commonly known for their use in facial recognition (Ahonen *et al.*, 2006), provide a powerful, robust, computationally efficient method for texture classification in image analysis (Ojala *et al.*, 2002). Under field conditions and with different image acquisition devices, illumination variability and color variability is high. The application of rotation invariant uniform LBPs to grayscale images could be useful for estimating alfalfa-grass stand composition under field conditions, because it is robust to changes in illumination and color (Ojala *et al.*, 2002).

Our objective was to develop a practical, farmer-accessible method that can be applied to estimate stand composition (i.e., grass and alfalfa DM fractions in binary mixes) under variable field conditions. We have previously tested multiple approaches including geometric pattern matching, color separation, blob detection, and tile extraction with fast Fourier transformation (combined with naïve Bayes classifier artificial intelligence and trained and untrained support vector machines) with unsatisfactory results (McRoberts *et al.*, 2012a). In this paper we develop a method combining digital image analysis using local binary patterns with statistical modeling to estimate alfalfa-grass stand composition. We describe the sampling process, local binary pattern method, and its implementation with several processing approaches to estimate stand composition.

2. Methods

2.1 Sampling

Mixed stands with different representative proportions of alfalfa and timothy grass (*Phleum pratense* L.) were identified in farmers' fields in Tompkins County, New York (42° 36′ N, 76° 30′ W) in spring 2011 (n=168, including 3 pure grass and 5 pure alfalfa samples). Representative samples were selected and delineated using a round hoop (66-cm diameter), which was rested on the vegetative canopy. A digital image was taken at 5-Megapixels resolution using an affordable, farmer-accessible, point-and-shoot digital camera (Canon PowerShot A3100IS) with automatic settings. A small bubble level was mounted on the camera LCD screen to ensure consistent image orientation parallel to the ground. Hoop edges were located as close to the image edges as possible and photos were taken freehand under natural field illumination conditions. Shadows were avoided in the image area. In each sample, alfalfa maximum height (maximum height of terminal bud), grass maximum height (maximum height of tallest extended grass blade), and grass canopy height were measured using a meter stick. Lighting conditions associated with each image were noted (e.g., full light or shade).

A 10-cm high quadrat was inserted under the hoop after photo acquisition to establish cutting height, and the hoop was lowered to ground level. Forage within each hoop (sample) was harvested at 10-cm above ground level by cutting at the height of the quadrat using battery-operated grass clippers. Harvested forage from each sample was manually separated into alfalfa and grass fractions. Plant species other than alfalfa and timothy grass were either absent or present only in trace quantities relative to alfalfa and timothy grass, and were discarded. Samples were dried to stable weight at 60°C in a forced-air oven. Samples were weighed directly after removal from the oven to determine alfalfa and grass dry fractions in each sample, calculated as *grass fraction = grass dry weight / (alfalfa dry weight + grass dry weight)*. Mean grass fraction for mixed samples was 0.413 with SD ± 0.148 (n=160).

2.2 Feature extraction, image filtering and pattern recognition

Images were cropped to remove the hoop and non-sampled area outside the hoop using the GNU Image Manipulation System (GIMP). The resulting ellipse from each original image was converted to grayscale using the derivation of luminance signal equation (Radiocommunication Sector of ITU, 2002) (Figure 1). Subsequent image processing was undertaken using the Scikit-Image library for Python (Van der Walt *et al.*, 2014).

Figure 1 Inner hoop area in mixed stand (alfalfa – grass) images was manually extracted from original image (left) in GIMP. The resulting color ellipse (center) was converted to grayscale (right) in Python.

Tile extraction (Polder *et al.*, 2007) divided the grayscale image into 64 x 64 pixel tiles for local processing (Figure 2). Larger and smaller tile sizes meeting the LBP operator criteria (power of two) were attempted, but were not practical for tile classification (e.g., too small to determine species in tile; both species simultaneously in tile due to large size). Images contained 462 tiles/image with SD ± 47.7 tiles/image. Tiles were manually classified as either mostly grass (1) or mostly alfalfa (0). Tiles with an equal amount of alfalfa and grass or material other than these species (e.g., bare ground, other species, unclear tile image areas) were considered unclassifiable, and were treated as missing data for classification purposes. Most tiles (78.4%) were successfully classified as alfalfa or grass. Out of 77,667 total tiles, 15,185 were classified as grass and 45,709 as alfalfa. Local binary patterns (Ojala *et al.*, 2002) were then extracted using SciPy Toolkit for Python (Jones *et al.*, 2001). Rotation invariant uniform LBPs effectively capture key information about stand composition in the image while minimizing sources of non-composition-related variation arising from lighting and color (Ojala *et al.*, 2002).

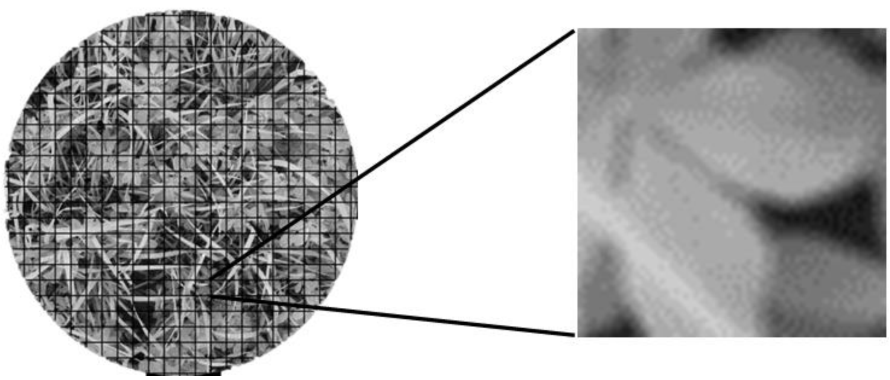

Figure 2 Tiles (64 x 64 pixels, right) were cropped from original images (left, with tile grid) for individual analysis.

2.3 Local binary pattern determination for individual pixels

Local binary patterns were determined for each individual pixel in each tile by comparing pixel grayscale value with a number of points (P) located equidistantly from each other on an arc of radius R from the central pixel (Ojala *et al.*, 2002). For pixels on or near tile edges, points lying outside the tile area were assigned a grayscale value of 0. Each point was assigned a 1 if its grayscale value exceeded or was equal to the central pixel and 0 if it was less than the central pixel. For example, with R=3, P=20, the resulting binary assignment for the 20 points generated 2^P possible patterns. We limited this to uniform rotation invariant local binary patterns with ≤ 2 bitwise transitions between 0 and 1, which accounted for the majority of patterns (Ojala *et al.*, 2002). There were *P – 1* uniform rotation invariant patterns with 2 transitions and two patterns with 0 transitions (all 1, all 0). Patterns with greater than 2 transitions were pooled into a final non-uniform pattern. In the case of *P*=20, there were 19 uniform rotation invariant patterns with 2 transitions, 2 patterns with 0 transitions, and one non-uniform pattern with > 2 transitions, for a total of 22 pattern values represented. Each central pixel was assigned a single value between 0 and 21 that represented its unique pattern. Continuing with the *P*=20 example, pattern 0 (all 0s) detected light spots while pattern 20 (all 1s) detected dark spots. Pattern 10 was a symmetric edge detector. Patterns between 0 and 10 and 10 and 20 were edges of varying curvature. The same interpretation applied to LBP pixel values assigned for each distinct *P*. Additional detail on the implementation of uniform rotation invariant LBPs is available in Ojala *et al.* (2002).

2.4 Local binary pattern histogram

Tiles contained 4096 pixels (64 x 64 pixels) and each pixel was assigned an LBP pattern value between 0 and 21 for the *P*=20 example. Thus, a histogram containing 22 frequencies described each tile. Each frequency was calculated by counting the total number pixels containing each pattern value for each of *j* bins, and reporting $\textit{pattern count in bin}_j / 4096$ as the frequency. For example, frequency 12 for a tile containing 328 pixels with a value of 12 would be $328 / 4096 = 0.07958984375$. Each frequency represented a fraction of total patterns expressed in the tile, and for each tile, $\sum \textit{LBP frequencies} = 1$.

2.5 R, P selection process from tile data

An iterative optimization process selected *R* and *P* using between 500 and 5000 randomly selected classified tiles for training (50% alfalfa and 50% grass) and 2000 different randomly selected classified tiles (50% alfalfa and 50% grass) for testing. Testing tiles were static for each iteration, which tested multiple *R* and *P* combinations with different training sets. Different *P* and *R* combinations were evaluated for each training set, with *R* allowed to vary between 1 and 4 and *P* between 2 and *R x 8* (practical upper limit for *P*) in increments of 2. For each testing tile, Kullback Leibler (KL) divergence was computed between the testing tile LBP histogram and each training tile's LBP histogram. Testing tiles were estimated according to the lesser of ∑*(KL divergence for alfalfa tiles)* and ∑*(KL divergence for grass tiles)* as alfalfa or grass, respectively. The *R, P* selection process was repeated 59 times to assess stability of selected values and to select *R, P* candidates for use in statistical model development (below). The *R, P* combination that most accurately estimated human tile classifications (according to *test tiles correctly classified / total test tiles*) was selected. Eight *R, P* sets emerged from this process (Table 1), and were used in statistical model development (below).

Table 1 Tile prediction accuracy rates (mean and SD) and number of times each local binary pattern operator pair (*R, P* combination) was selected (*n*), sorted by accuracy rate in descending order for *R, P* sets.

R	*P*	*n*	Accuracy rate	
			Mean	SD
2	16	12	0.729	0.0119
2	12	6	0.726	0.0070
1	8	20	0.725	0.0112
4	32	6	0.723	0.0081
3	24	7	0.723	0.0082
3	20	4	0.723	0.0110
4	28	2	0.722	0.0049
4	24	2	0.716	0.0046

2.6 Generation of stand composition estimate from local binary pattern data

We implemented three statistical modeling approaches to estimate stand composition (grass fraction response) from LBP data. Statistical modeling was undertaken in SAS for Windows 9.3 (SAS Institute Inc., 2011). Pure alfalfa and pure grass samples were excluded from model development.

2.6.1 Model development and testing on tile local binary pattern bin average for whole images

The LBP tile data were reduced to a single histogram for each original hoop image by calculating the average value of each LBP frequency for each *R, P* combination. Candidate predictors in models estimating grass fraction (GFRAC) included average LBP histogram frequencies from each *R, P* set (bin_0 through bin_{p+1}), grass canopy height (GCPY), grass maximum height (GMAX) and alfalfa maximum height (AMAX). Regression model selection for GFRAC response was undertaken in PROC GLMSELECT using stepwise selection (SAS Institute Inc., 2011) (model selection based on *PRESS* statistic) with all candidate predictors, according to the model $y = X\beta + \varepsilon$. For each image i,

$$GFRAC_{rpi} = \beta_0 + \beta_{h_0} bin_0 + \beta_{h_1} bin_1 + \cdots + \beta_{h_p} bin_p + \beta_{h_{p+1}} bin_{p+1} + \beta_2 GCPY + \beta_3 GMAX + \beta_4 AMAX$$

Models selected by PROC GLMSELECT were tested for multicollinearity in PROC REG, and further reduced to ensure variance inflation factors near or below 10. Residuals and Q-Q plots were visually examined for normality. Model fit was evaluated based on lowest prediction sum of squares (*PRESS*) statistic and root mean squared error (RMSE), and highest R^2 and R^2_{pred}. PRESS is useful to assess model fit in cross-validation, and is calculated as $\sum_{i=1}^{n}(y_i - f_i)^2$, where n is number of observations, y_i is the actual value of the ith data point, and f_i is the predicted value for y_i from a model trained using all data except the ith observation (Meyers, 1990). The PRESS statistic is the sum of squares of prediction residuals for observations not used to fit the model. The R^2_{pred} is always lower than R^2, because it accounts for model prediction capability according to: $R^2_{pred} = 1 - \frac{PRESS}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$ (Meyers, 1990). The dataset was not split for training and testing in this first-stage assessment of LBP methods for estimation of mixed stand composition. Top *R, P* sets were tested for performance differences in GFRAC estimation based on lighting conditions (full light or shade) by adding a binary covariate.

2.6.2 Tile model development and testing combined with whole image model development and testing

The two-phase model selection process first involved developing tile models to estimate tile probability of grass from local binary pattern bins. Second, average probability of grass, considered a grass coverage estimate for whole hoop images, was calculated and used as the key predictor in whole image model selection. Whole image models estimated grass fraction from the LBP-generated grass coverage estimate (from tile model), alfalfa maximum height and grass canopy height.

In the tile model, a binary response was defined by classified tile values (alfalfa = 0, grass = 1). Unclassified tiles were treated as missing data and ignored in analysis. Tile LBP models were selected using PROC GLIMMIX (SAS Institute Inc., 2011), with binomial distribution and logit link function, using LBP frequencies extracted from each *R, P* set (Table 1), according to the model $log(\frac{p_{grass}}{1-p_{grass}}) = X\beta + Zb + \varepsilon$, where p_{grass} is the probability a tile is grass, X is a matrix of fixed-effect predictors, β is a vector of regression coefficients for fixed effects, Z is a random effects matrix, b is a vector of random effects, and ε is a vector of residuals. Fixed effects included LBP frequencies for each tile (observation). Photo ID was included as a random effect to account for non-independence of tiles within photos. Tile model evaluation criteria included *maximum likelihood*, calculated as $\sum(classification * \log(\widehat{p_{grass}}) + (1 - classification) * \log(1 - \widehat{p_{grass}}))$, where *classification* is human classified value for each tile (alfalfa (0) or grass (1)) and $\widehat{p_{grass}}$ is model-estimated probability of grass for each tile. This likelihood approach was chosen to assess model fit rather than classification error rate, because it does not require a specific cutoff (e.g., 0.5), which is important in our dataset due to mean grass fraction < 0.5 (and fewer tiles classified as grass than alfalfa), resulting in p_{grass} weighted toward alfalfa. This likelihood approach favors models predicting tile p_{grass} closer to 0 and 1 over less certain models (e..g, $p_{grass} = 0.5$). Model-predicted p_{grass} data were generated for all tiles in each *R, P* combination, and mean of tile-predicted p_{grass} for whole hoop images was used as a key predictor in whole image model selection. Performance of this parameter in predicting actual stand composition was also considered in selecting most effective tile models (and *R, P* combinations). Three approaches to tile model development were employed: (1) selection from bins representing light spots, symmetric edges, dark spots and non-uniform patterns, (2) selection from all significant histogram bins, and (3) selection from bins suggested by recursive partitioning in JMP 11.2.0 (SAS Institute Inc., 2013).

Pairwise correlations were evaluated between whole image GFRAC and LBP coverage estimates predicted by selected tile models for 8 *R, P* combinations (Table 1). Whole image model selection was undertaken in PROC GLM (SAS Institute Inc., 2011) with GFRAC continuous response. Fixed effects included LBP coverage estimate (mean p_{grass}), AMAX and GCPY. Whole image models selected using the tile model-generated p_{grass} estimate did not include interactions or quadratic effects for ease of cross-model comparison. Model fit was evaluated using the criteria outlined in Section 2.5.1. Final tile model selection was based on performance of whole image models containing tile overall p_{grass} estimate.

2.6.3 Model development and testing on whole image local binary pattern bins

Further image extraction was required to process whole images (in lieu of tiles), because processing image units must be a power of 2 pixels. The closest match was 2^{10} for whole images, thus a 1024 by 1024 pixel square was extracted from inside hoop images (approximately 60% of total hoop area). The LBP histograms were extracted from whole images (rather than individual tiles) as described in section 2.3 and 2.4 by manually testing *R, P* combinations between 1, 4 and 7, 56. Statistical model selection was undertaken as in Section 2.5.1.

3. Results

3.1 Relationship between classified tiles and grass fraction

Grass coverage estimates generated by human tile classification underestimated actual GFRAC (as measured by dry biomass) in a direct relationship, particularly for low- and mid-range grass fractions (Figure 3). Samples near GFRAC values of 0.5 contained higher proportion of unclassified tiles in original hoop images (Figure 3).

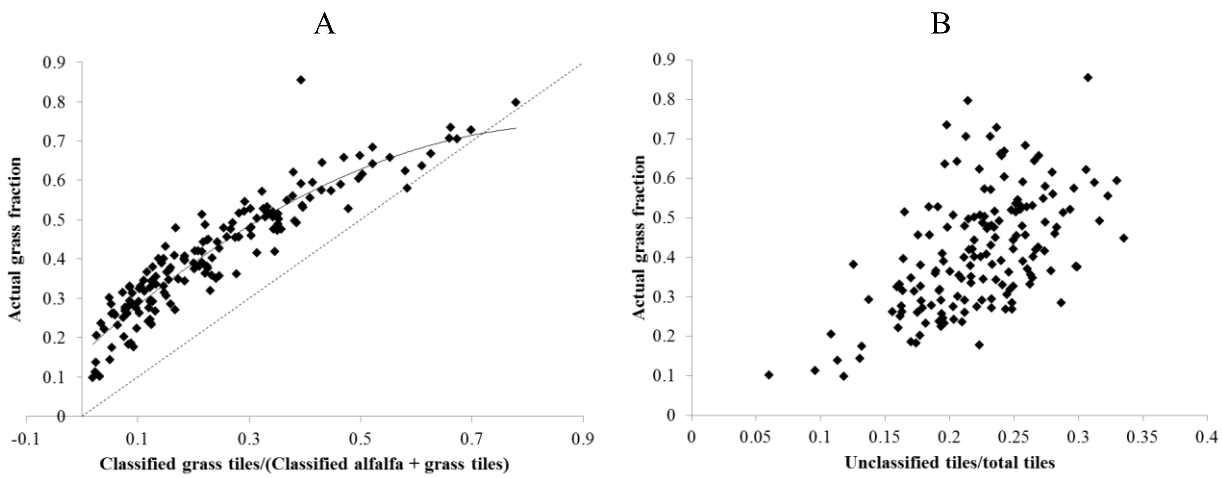

Figure 3 Quadratic relationship between actual grass fraction and fraction estimated by tile classification (A, *n*=160), described by $y = -0.7266x^2 + 1.302x + 0.159$ ($r^2 = 0.872$). Dashed line is 1:1 relationship. Relationship between actual grass fraction and fraction unclassified tiles in each original image (B, *n*=160).

3.2 Local binary patterns for alfalfa and grass differentiation

Symmetric edge detection bins were most significant and most effective at partitioning tiles into alfalfa and grass (Table 2, Figure 4, Appendix 1). Average symmetric edge proportion of total patterns in each tile was significantly higher for grass tiles than alfalfa tiles. Other patterns also exhibited mean differences, but average proportion of total patterns differed by a smaller margin for other bins. For example, average proportion of total patterns expressed for light spots, dark spots, and non-uniform patterns was higher for alfalfa tiles than grass tiles. Consequently, uniform patterns were expressed in greater average proportion for grass tiles, which was driven by the symmetric edge bin and others near it (Table 2). Bins neighboring the symmetric edge detector generally had higher grass fractions than alfalfa fractions, although more bins adhered to this trend above $P/2$ than below it. Conversely, bins neighboring light spots (P=0) and dark spots (P=P) tended to be expressed in higher proportion for alfalfa tiles than grass tiles.

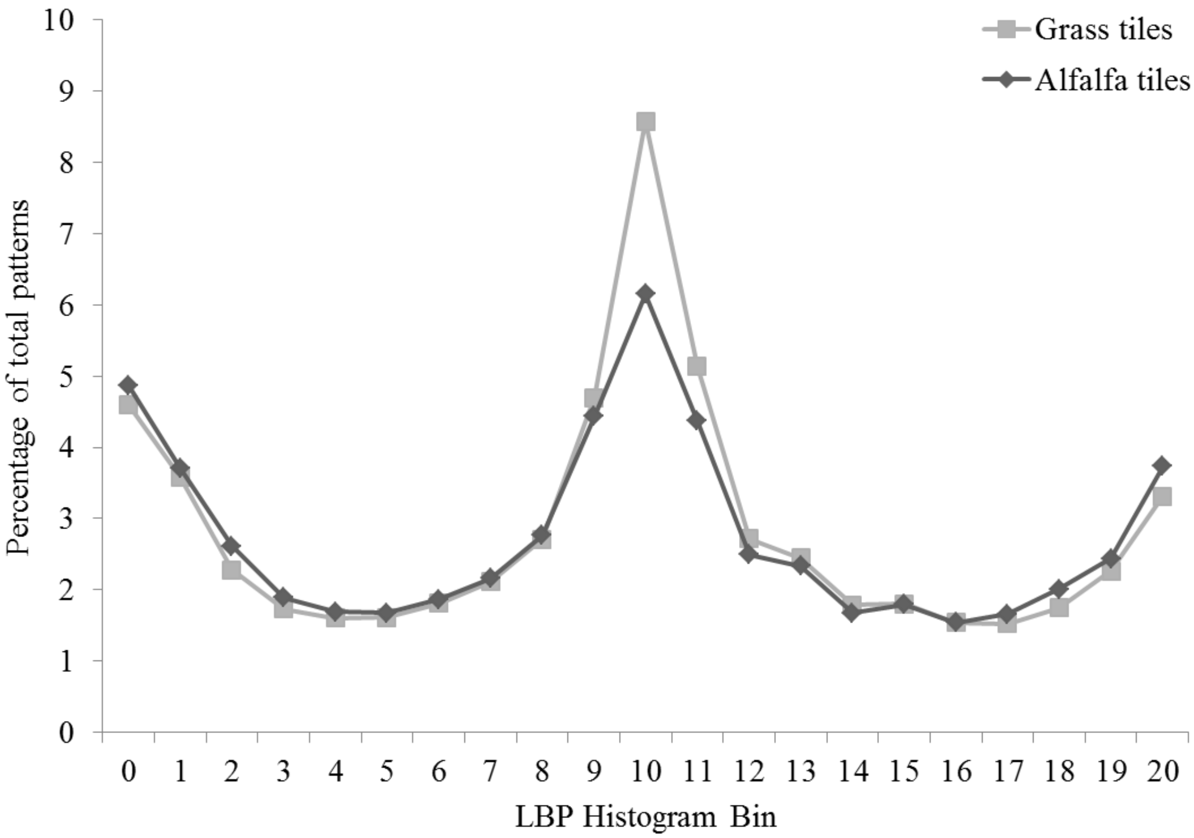

Figure 4 Average percentage of total local binary patterns in dataset for grass tiles (gray line, n=15,185) and alfalfa tiles (black line, n=45,709) for R=3, P=20. Bin 0 represents lights spots, bins 1 to 9 and 11 to 19 edges of varying curvature, bin 10 symmetric edges, and bin 20 dark spots. Bin 21 (not shown) represents non-uniform patterns, and contained 42.1% of alfalfa tile patterns and 40.4% of grass tile patterns. Relationship around the symmetric edge detector bin and interpretation are similar for other R, P sets.

Table 2 Mean differences between average percentage of total local binary patterns in each histogram bin for grass tiles (n=15,185) and alfalfa tiles (n=45,709) in R, P sets. Bins where grass mean > alfalfa mean are positive, and are highlighted in gray. Bin P+1 is percentage non-uniform patterns. Bin P represents dark spots. Bin 0 represents lights spots. Bins 1 to P/2 – 1 and P/2 +1 to P-1 are edges of varying curvature.

Bin	Mean difference for R, P sets (Grass % - Alfalfa %)							
	1,8	2,12	2,16	3,20	3,24	4,24	4,28	4,32
0	0.25%	-0.26%	-0.23%	-0.27%	-0.26%	-0.28%	-0.23%	-0.23%
1	-0.04%	-0.19%	-0.05%	-0.12%	-0.04%	-0.17%	-0.10%	-0.04%
2	-0.34%	-0.44%	-0.36%	-0.33%	-0.30%	-0.28%	-0.29%	-0.28%
3	-0.48%	-0.25%	-0.30%	-0.16%	-0.20%	-0.07%	-0.12%	-0.17%
4	1.55%	-0.26%	-0.21%	-0.09%	-0.10%	-0.01%	-0.03%	-0.06%
5	-0.66%	-0.02%	-0.20%	-0.07%	-0.07%	0.00%	-0.01%	-0.02%
6	-0.61%	2.81%	-0.21%	-0.06%	-0.06%	0.00%	-0.01%	-0.01%
7	-0.04%	0.45%	0.26%	-0.05%	-0.05%	0.00%	0.00%	-0.01%
8	0.16%	0.00%	2.45%	-0.06%	-0.04%	0.02%	0.00%	-0.01%
9	0.21%	-0.18%	0.63%	0.26%	-0.05%	0.04%	0.01%	0.00%
10		-0.38%	0.06%	2.43%	-0.05%	0.01%	0.03%	0.01%
11		-0.23%	-0.07%	0.77%	0.40%	0.21%	0.03%	0.02%
12		-0.46%	-0.11%	0.22%	2.20%	2.11%	0.00%	0.03%
13		-0.59%	-0.24%	0.11%	0.83%	0.73%	0.32%	0.02%
14			-0.30%	0.11%	0.25%	0.25%	1.95%	-0.01%
15			-0.09%	0.00%	0.09%	0.21%	0.77%	0.40%
16			-0.47%	0.00%	0.14%	0.15%	0.27%	1.79%
17			-0.57%	-0.13%	0.03%	0.10%	0.16%	0.77%
18				-0.26%	0.05%	0.10%	0.16%	0.29%
19				-0.17%	0.02%	0.03%	0.07%	0.13%
20				-0.42%	-0.06%	0.05%	0.13%	0.18%
21				-1.73%	-0.17%	-0.05%	0.05%	0.06%
22					-0.22%	-0.18%	0.07%	0.12%
23					-0.11%	-0.17%	0.05%	0.11%
24					-0.41%	-0.27%	0.00%	0.07%
25					-1.80%	-2.51%	-0.09%	0.01%
26							-0.18%	0.06%
27							-0.13%	0.02%
28							-0.28%	-0.04%
29							-2.59%	-0.12%
30								-0.16%
31								-0.09%
32								-0.28%
33								-2.58%

Bin 2 exhibited greater separation of alfalfa and grass than neighboring bins. Bins representing edges of varying curvature generally had low numerical mean differences between bin 0 and bin *P*/2 – 2, and bin *P*-1 and bin *P*/2+3 (alfalfa > grass), and higher differences near the symmetric edge detector (grass > alfalfa). The lowest *R, P* combination (1,8) differed slightly, due to smaller number of possible patterns, and was less effective in separating alfalfa and grass than other *R, P* sets tested.

3.3 Field measurements

Grass fraction was more highly correlated with grass canopy height (r=0.38) than grass maximum height (r=0.21) and alfalfa maximum height (r=0.06). Grass maximum height was not included as a candidate covariate in grass fraction models, because it usually dropped from models that included grass canopy height.

3.4 Model development on tile bin averages for whole images

Grass fraction models were selected individually from tile LBP means for *R, P* combinations, as well as GCPY and AMAX for original hoop images (Table 3). Coefficient of determination (R^2) ranged from 0.50 to over 0.86, R^2_{pred} from 0.47 to 0.85 and calibration RMSE from 0.10 to 0.057. Top *R, P* combinations based on minimum PRESS were 4,32; 3,24; and 3,20. Top models generally had higher *R, P* combinations and were those selected from automated model selection with post-selection manual adjustments to prevent multicollinearity (rather than just from bins representing light spots, dark spots, symmetric edges, and non-uniform patterns). All models contained the symmetric edge detector bin. Bin 0 (light spots) was frequently selected, except in top tier automated selection models.

Table 3 Models estimating grass fraction developed using tile local binary pattern bin means, grass canopy height and alfalfa maximum height for whole images. Each row represents a single model (*n*=160), and two models are presented for each *R*, *P*. Models were evaluated based on PRESS statistic, R^2, R^2_{pred}, and *RMSE*, and sorted in ascending order by PRESS. Model effect representing symmetric edges is in bold for each model.

R	*P*	R^2	*PRESS*	R^2_{pred}	*RMSE*	Model effects
4	32	0.859	0.534	0.847	0.0566	bin2 **bin16** bin19 bin27 AMAX
3	24	0.855	0.560	0.840	0.0577	bin10 **bin12** bin19 bin22 GCPY AMAX
3	20	0.851	0.563	0.839	0.0582	[a] bin0 **bin10** bin21 GCPY AMAX
3	20	0.852	0.571	0.837	0.0582	bin3 bin7 **bin10** bin18 GCPY AMAX
2	12	0.849	0.575	0.836	0.0585	bin5 **bin6** bin11 GCPY AMAX
2	16	0.848	0.580	0.834	0.0589	bin0 **bin8** bin15 bin17 GCPY AMAX
3	24	0.843	0.596	0.830	0.0598	[a] bin0 **bin12** bin25 GCPY AMAX
4	28	0.842	0.598	0.829	0.0600	bin12 **bin14** bin23 bin26 AMAX
2	12	0.840	0.604	0.827	0.0603	[a] bin0 **bin6** bin13 GCPY AMAX
2	16	0.832	0.638	0.818	0.0619	[a] bin0 **bin8** bin16 bin17 GCPY AMAX
4	24	0.823	0.662	0.811	0.0626	bin4 bin7 **bin12** bin14 bin20 GCPY AMAX
4	32	0.817	0.705	0.798	0.0647	[a] bin0 **bin16** bin32 bin33 GCPY AMAX
4	28	0.813	0.724	0.793	0.0655	[a] bin0 **bin14** bin28 bin29 GCPY AMAX
4	24	0.805	0.754	0.784	0.0668	[a] bin0 **bin12** bin24 bin25 GCPY AMAX
1	8	0.522	1.782	0.491	0.104	[a] bin0 **bin4** GCPY AMAX
1	8	0.517	1.825	0.478	0.105	bin3 **bin4** bin6 GCPY AMAX

[a] Model selected only from bins representing symmetric edges, light spots, dark spots, and non-uniform patterns.

3.5 Combined tile model development and whole image model development from tile model-generated grass coverage estimate

Maximum likelihood for tile models ranged from -31,000 to -19,000, and tended to favor less parsimonious models within each *R, P*, and higher *R, P* combinations (Table 4). The top tile models based on maximum likelihood were 4,32; 4,28; and 4,24. Maximum likelihood-selected tile models differed from models selected based on performance (low *PRESS*) in whole image models (Table 4). Coefficient of determination in whole image models ranged from 0.2 to over 0.82, R^2_{pred} from 0.2 to 0.81 and calibration RMSE from 0.13 to 0.064. Top *R, P* based on *PRESS* were 3,24; 3,20; 4,32; and 4,28. Top models were generally selected from higher *R*, and *P* in the 20s. Models were selected three ways for each *R, P*, and models fueled by bins suggested in recursive partitioning regression generally performed better than those built based on meaningful bins (symmetric edge, light spot, dark spot, non-uniform patterns) or constructed using all significant bins. All tile models contained the symmetric edge detector bin. Bins neighboring symmetric edge detector bin and bin 2 were also frequently represented in models selected from bins identified with recursive partitioning. The binary lighting effect was only significant in combined whole image model selection in models with all significant bins, and was not included in models (Table 4).

3.6 Model development on whole hoop image local binary pattern bins

Whole image models selected from LBP bins extracted from whole hoop images did not perform as well as other approaches tested above. The GFRAC models were selected using automated model selection procedures for more than 50 *R, P* sets from LBP bins, AMAX and GCPY, with final models adjusted to eliminate multicollinearity. Fourteen top models were selected based on $PRESS < 1.10$, $RMSE < 0.08$, and $R^2_{pred} > 0.69$ (Table 5). Additional models selected using only key bins (e.g., light spots, dark spots, symmetric edges, and non-uniform patterns) did not outperform those in Table 5.

Table 4 Tile model criteria included maximum likelihood (ML), performance of tile model-generated grass coverage estimates in whole image models with alfalfa maximum height and grass canopy height covariates (based on R^2, *PRESS*, R^2_{pred}, and *RMSE*), and bivariate correlation of tile model-generated grass coverage estimates with grass fraction in dry biomass in whole hoop images (GFRAC corr (*r*)). Symmetric edge effect is bold for each model.

R	*P*	Tile model (*n*=60,894 classified tiles)		GFRAC corr (*r*)	Whole image model (n=160 images)			
		ML	Model effects		R^2	*PRESS*	R^2_{pred}	*RMSE*
3	24	-20893	[c] **bin12** bin25 bin11 bin10	0.895	0.817	0.676	0.807	0.0641
3	20	-20374	[c] **bin10** bin9 bin21 bin2	0.889	0.810	0.700	0.800	0.0652
3	20	-19467	[b] bin0 bin1 bin2 bin7 bin8 bin9 **bin10** bin11 bin12 bin13 bin14 bin15 bin17 bin18 bin20	0.886	0.805	0.718	0.795	0.0661
4	32	-19457	[c] **bin16** bin15 bin14 bin2 bin3	0.884	0.800	0.737	0.789	0.0669
4	28	-19566	[c] **bin14** bin13 bin12 bin2	0.885	0.800	0.738	0.789	0.0669
4	24	-18800	[b] bin0 bin1 bin2 bin7 bin8 bin9 bin10 bin11 **bin12** bin13 bin14 bin15 bin17 bin18 bin19 bin20 bin22 bin23 bin24	0.882	0.794	0.760	0.783	0.0679
2	16	-22550	[c] **bin8** bin6 bin17	0.876	0.794	0.761	0.783	0.0680
3	24	-19242	[b] bin0 bin1 bin2 bin3 bin4 bin6 bin8 bin9 bin10 bin11 **bin12** bin13 bin14 bin15 bin16 bin17 bin20 bin21 bin22 bin24	0.879	0.794	0.761	0.782	0.0680
2	12	-22022	[c] bin2 bin 4 bin5 **bin6** bin13	0.879	0.792	0.768	0.780	0.0682
3	20	-22293	[a] bin0 **bin10** bin20 bin21	0.872	0.790	0.778	0.778	0.0687
4	32	-18338	[b] bin0 bin1 bin2 bin3 bin9 bin11 bin12 bin13 bin14 bin15 **bin16** bin17 bin18 bin19 bin20 bin21 bin22 bin23 bin25 bin26 bin27 bin29 bin30 bin31 bin32	0.878	0.788	0.779	0.777	0.0689
4	24	-19765	[c] **bin12** bin11 bin2	0.878	0.789	0.780	0.777	0.0687
4	28	-18671	[b] bin0 bin1 bin2 bin3 bin8 bin9 bin10 bin11 bin12 bin13 **bin14** bin15 bin16 bin17 bin18 bin19 bin20 bin21 bin23 bin26 bin27 bin28	0.878	0.787	0.786	0.775	0.0691
3	24	-22280	[a] bin0 **bin12** bin24 bin25	0.870	0.787	0.789	0.774	0.0691
2	16	-22753	[a] bin0 **bin8** bin16 bin17	0.853	0.764	0.868	0.752	0.0727
2	16	-21516	[b] bin0 bin1 bin2 bin3 bin4 bin5 bin6 bin7 **bin8** bin9 bin10 bin12 bin13 bin14 bin15	0.858	0.765	0.868	0.752	0.0726
2	12	-21557	[b] bin0 bin1 bin2 bin3 bin4 bin5 **bin6** bin7 bin8 bin10 bin11	0.841	0.738	0.967	0.724	0.0766
4	32	-23379	[a] bin0 **bin16** bin32 bin33	0.840	0.733	0.987	0.718	0.0774
4	28	-23478	[a] bin0 **bin14** bin28 bin29	0.838	0.729	1.00	0.714	0.0779
2	12	-22833	[a] bin0 **bin6** bin12 bin13	0.824	0.723	1.02	0.709	0.0789
1	8	-26375	[a] bin0 **bin4** bin8 bin9	0.655	0.529	1.74	0.502	0.1028
4	24	-25255	[a] bin0 **bin12** bin24	0.687	0.523	1.77	0.494	0.1034
1	8	-28852	[b] bin6 bin2 **bin4** bin9	0.328	0.290	2.61	0.253	0.1261
1	8	-31058	[c] bin2 **bin4** bin6	0.165	0.238	2.81	0.198	0.1307

[a] Model selected from symmetric edge, light spot, dark spot, and non-uniform pattern bins.
[b] All significant LBP bins were selected.
[c] Model selected from bins identified using recursive partitioning (SAS Institute Inc., 2013).

Table 5 Models estimating grass fraction were selected using local binary pattern bins extracted from whole images for different *R* and *P* combinations, as well as grass canopy height and alfalfa maximum height. Each row represents a single model (n=160). Models were evaluated based on *PRESS* statistic, R^2, R^2_{pred}, and *RMSE*, and sorted in ascending order by *PRESS*. Model effect representing symmetric edges is in bold.

R	*P*	R^2	*PRESS*	R^2_{pred}	*RMSE*	Model effects
6	48	0.765	0.976	0.721	0.0747	bin0 bin2 bin3 bin12 bin14 bin22 **bin24** bin37 bin41 bin48 GCPY AMAX
7	56	0.751	0.985	0.718	0.0760	bin2 bin10 bin26 bin29 bin40 bin56 GCPY AMAX
6	46	0.744	1.045	0.701	0.0778	bin3 bin7 bin9 bin12 bin13 bin21 **bin23** bin31 bin46 GCPY AMAX
7	50	0.733	1.049	0.700	0.0786	bin0 bin2 bin24 **bin25** bin49 bin50 GCPY AMAX
5	36	0.732	1.059	0.697	0.0788	bin2 bin10 bin17 **bin18** bin24 bin36 GCPY AMAX
5	40	0.730	1.067	0.695	0.0790	bin2 bin11 bin19 **bin20** bin28 bin40 GCPY AMAX
5	32	0.726	1.069	0.694	0.0794	bin2 bin15 **bin16** bin23 bin32 GCPY AMAX
7	52	0.731	1.069	0.694	0.0792	bin2 bin12 bin14 bin25 **bin26** bin50 bin52 GCPY AMAX
3	20	0.725	1.077	0.692	0.0795	bin1 bin7 bin9 **bin10** bin14 GCPY AMAX
5	38	0.728	1.078	0.692	0.0794	bin2 bin9 bin18 **bin19** bin28 bin38 GCPY AMAX
5	28	0.731	1.079	0.692	0.0793	bin1 bin2 bin7 bin13 **bin14** bin20 bin28 GCPY AMAX
4	32	0.724	1.081	0.691	0.0796	bin11 bin15 **bin16** bin24 bin32 GCPY AMAX
2	14	0.718	1.086	0.690	0.0803	bin5 **bin7** bin11 bin12 GCPY AMAX

4. Discussion

Clear differentiation of local binary patterns in alfalfa and grass tiles is the principal reason that this method is effective in estimating grass coverage and grass fraction of dry matter from digital images representing mixed stand samples. Curved alfalfa leaves in tiles classified as alfalfa contain fewer symmetric edges and more non-uniform patterns relative to tiles with predominantly grass (more long, straight or slightly curving leaf edges). Thus, the symmetric edge detector bin is most effective in differentiating grass and alfalfa in our dataset. Similarly, light spots, dark spots, and edges of higher curvature are present in higher fractions for alfalfa tiles, while edges closer to symmetric tend to be more prevalent in grass tiles regardless of *R, P* combination. Those farther from the symmetric edge detector are alfalfa, especially above the symmetric edge detection bin. Bin 2 also emerged frequently as a significant effect in tile models, and exhibited greater alfalfa-grass differentiation capacity than neighboring bins. Bin 2 represents slight curves close to light spots, which are more prevalent in alfalfa than grass.

Manual tile classification tended to underestimate actual grass fraction in a direct quadratic relationship, particularly for low- and mid-range grass fractions (Figure 3). This could be attributed to broader alfalfa leaves obscuring grass leaves in area viewed, and the lack of correction for alfalfa and grass height relationships. This is consistent with the linear relationship between actual grass fraction and researcher visual estimation of grass fraction for nearly 600 samples in 2004 (y = 0.22 + 0.69x, R^2=0.43, *RMSE*= 0.147) (McRoberts *et al.*, 2012a), which underestimated grass fraction for low- and mid- range fractions. Furthermore, the positive relationship in our dataset between actual grass fraction and proportion unclassified tiles up to GFRAC = 0.5 suggests that human classification difficulty increases when stands are highly mixed. Tiles would be more likely to contain both alfalfa and grass, which precludes binary classification in these cases.

Grass fraction models selected from LBP tile bin means for whole hoop images are most promising for GFRAC estimation, followed closely by combined tile model selection and whole hoop model selection. Consistent with most accurate *R, P* sets in the automated *R, P* selection procedure (Table 1), leading *R, P* candidates in both model selection approaches are 3,24; 3,20; and 4,32, although other models from high *R* (3 or 4) and high *P* (20 to 24 for *R*=3 and 28 to 32 for *R*=4) performed nearly as well based on low *PRESS* and other statistical selection factors considered. Leading models contain the symmetric edge detector, at least one neighboring bin, one bin near light spots, and often a bin between symmetric edges and dark spots.

Local binary patterns extracted directly from whole images were the least effective in estimating grass fraction in mixed stands in our dataset. This can be partly attributed to the image subsample required by the LBP operator (representing about 60% of whole hoop image area). Similar bins emerged for whole images as in tile models, and often included bin 0 (light spots), bin 2, symmetric edge detector, and bin *P* (dark spots). However, it is unlikely that whole image models would perform as well as tile models due to the scale of data analyzed. Furthermore, *R, P* selection is arbitrary and manual using this method due to lack of binary classified response feasibility for whole hoop images, which precludes automated *R, P* selection similar to the approach used with classified tiles.

Similar to facial recognition, smaller image sections in mixed stands may contain relationships that permit more effective pattern recognition upon aggregation. We chose tile size (64 x 64 pixels) so that tiles could be effectively classified by humans. Larger and smaller regions were tested, but presented complications due to areas too small to classify or large areas that usually contained both species. Analysis of local areas (tiles) in our study is more effective than whole image analysis with LBPs. This is consistent with Ahonen *et al.* (2004) facial recognition tests, which indicated that subdivision of images into smaller rectangular regions improved performance.

Images represented a range in illumination and color, but lighting did not contribute to most GFRAC estimation models. Local binary patterns are more robust than other methods to changes in illumination and color, such as those experienced under field conditions, due to their gray scale invariance (Ojala *et al.*, 2002). This is an important reason that the LBP approach is favorable relative to other potential automated approaches for estimating mixed stand composition under field conditions. Promising alternative approaches requiring further field testing include erosion and dilation procedures (Himstedt *et al.*, 2009, 2010, 2012) and canopy reflectance (Post *et al.*, 2007). Aside from local binary patterns, erosion and dilation procedures may have the most potential for field use, although effectiveness would decrease for grass species with wider leaves in mixed stands, because they would be less likely to drop out completely during the erosion process. We undertook preliminary testing of other less effective approaches including tile extraction and fast Fourier transform (Polder *et al.*, 2007). These approaches had little alfalfa – grass differentiation capacity in the absence of species height relationships (McRoberts *et al.*, 2012a; McRoberts *et al.*, 2012b). More complex LBP processing approaches such as support vector machines could be considered.

Accurate grass fraction estimation with LBPs in digital images is promising based on this study. The current dataset contained images from a single grass species, single sampling year, and single camera. Development of GFRAC prediction equations will require training and testing on a more diverse dataset. If successful, resulting equations will contribute to improved alfalfa-grass harvest management and economic outcomes for dairy farms in the northeast U.S. by reducing uncertainty in existing NDF prediction equations. Equations could be built into a web program and smartphone app requiring users to upload representative digital images from mixed alfalfa-grass fields and to input species height measurements. The program would return estimates of botanical composition (GFRAC), NDF concentration, and optimal harvest date (based on target NDF and estimated daily change in NDF given weather conditions until harvest). Our GFRAC estimation approach uses technology commonly available to farmers and consultants (digital or smartphone camera), permitting widespread use without investment in additional infrastructure. Such a tool allows farmers and consultants to prioritize the order of harvest of alfalfa-grass fields to increase the odds of obtaining optimal forage NDF for lactating dairy cow diets.

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APPENDIX 1: Percentage of total local binary patterns in histogram bins for grass and alfalfa

Table A1 Average percentage of total local binary patterns in each histogram bin differed for grass tiles (n=15,185) and alfalfa tiles (n=45,709) in each *R, P* set. Symmetric edge detection bins are in bold. Bin *P*+1 is percentage non-uniform patterns. Bin *P* represents dark spots. Bin 0 represents lights spots. Bins 1 to *P*/2 – 1 and *P*/2 +1 to P-1 are edges of varying curvature.

Bin	*R, P* Alfalfa (%), Grass (%)															
	1,8		2,12		2,16		3,20		3,24		4,24		4,28		4,32	
	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
0	4.97	5.22	5.17	4.91	5.93	5.70	4.86	4.59	5.07	4.81	4.23	3.95	4.66	4.43	4.75	4.52
1	8.13	8.10	5.36	5.17	4.09	4.04	3.71	3.59	3.04	3.01	3.54	3.36	2.97	2.87	2.53	2.49
2	5.36	5.01	3.74	3.30	3.29	2.93	2.61	2.28	2.43	2.13	2.16	1.87	2.20	1.91	2.09	1.81
3	11.4	10.9	3.24	3.00	2.76	2.46	1.89	1.73	1.83	1.63	1.48	1.41	1.52	1.40	1.50	1.34
4	**21.3**	**22.8**	3.98	3.73	2.51	2.30	1.69	1.60	1.53	1.43	1.27	1.26	1.25	1.22	1.18	1.12
5	14.4	13.7	6.76	6.74	2.95	2.74	1.68	1.61	1.45	1.37	1.21	1.21	1.16	1.15	1.07	1.05
6	7.77	7.16	**11.4**	**14.2**	3.79	3.59	1.87	1.81	1.42	1.36	1.20	1.19	1.12	1.11	1.00	1.00
7	7.51	7.46	7.45	7.90	6.29	6.55	2.16	2.11	1.56	1.51	1.32	1.32	1.10	1.09	0.974	0.967
8	7.54	7.70	4.10	4.10	**9.20**	**11.6**	2.77	2.71	1.73	1.69	1.41	1.43	1.17	1.17	0.955	0.949
9	11.6	11.9	3.79	3.60	6.73	7.36	4.43	4.70	2.04	1.99	1.65	1.69	1.26	1.27	1.02	1.02
10			3.65	3.27	3.74	3.80	**6.15**	**8.58**	2.61	2.56	2.07	2.09	1.40	1.43	1.09	1.10
11			4.61	4.38	3.30	3.23	4.37	5.14	4.10	4.51	3.21	3.42	1.62	1.65	1.19	1.20
12			5.11	4.65	2.52	2.41	2.50	2.72	**5.29**	**7.49**	**4.09**	**6.20**	2.05	2.05	1.30	1.32
13			31.6	31.0	2.65	2.40	2.34	2.45	4.06	4.88	3.00	3.73	3.08	3.40	1.53	1.56
14					2.94	2.64	1.68	1.79	2.33	2.57	1.75	2.00	**3.67**	**5.62**	1.95	1.94
15					2.98	2.89	1.80	1.80	2.18	2.28	1.60	1.81	2.85	3.62	2.86	3.26
16					5.50	5.03	1.54	1.54	1.56	1.70	1.18	1.33	1.64	1.92	**3.26**	**5.05**
17					28.8	28.3	1.66	1.53	1.57	1.61	1.37	1.46	1.70	1.86	2.68	3.46
18							2.01	1.75	1.34	1.39	1.01	1.11	1.16	1.32	1.54	1.83
19							2.44	2.26	1.35	1.36	1.20	1.23	1.30	1.37	1.59	1.72
20							3.73	3.31	1.34	1.27	1.05	1.11	0.99	1.12	1.10	1.28
21							42.1	40.4	1.53	1.36	1.17	1.12	1.04	1.09	1.17	1.23
22									1.77	1.56	1.48	1.29	0.96	1.03	0.954	1.07
23									1.88	1.77	2.01	1.84	1.00	1.06	0.897	1.01
24									3.85	3.44	2.86	2.58	1.00	1.00	0.813	0.886
25									41.1	39.3	51.5	49.0	1.15	1.06	0.986	1.00
26													1.41	1.23	0.853	0.910
27													1.61	1.48	0.878	0.900
28													3.11	2.82	0.926	0.890
29													48.8	46.3	1.08	0.97
30															1.29	1.13
31															1.32	1.23
32															3.15	2.87
33															48.5	45.9

CHAPTER 5: LOW-INFRASTRUCTURE FILTER BAG TECHNIQUE FOR NEUTRAL DETERGENT FIBER ANALYSIS OF FORAGES

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A supplementary instructional video associated with the article is available in Appendix A of the online version at: http://dx.doi.org/10.1016/j.anifeedsci.2013.09.007.

Abstract

We tested a water bath filter bag technique (WB) for analysis of neutral detergent fiber (NDF) versus a standard filter bag technique (ANKOM). The principal difference between WB and ANKOM was absence of a pressurized chamber for WB. The NDF method we used (aNDF) did not include sodium sulfite. One-hundred and ninety-six diverse forage and silage samples were gathered from Vietnam and New York State including: 40 C_3 grasses, 122 C_4 grasses, 21 legumes and 13 silages. Samples were completely randomized for parallel processing in ANKOM and WB. Water bath aNDF levels were strongly correlated with ANKOM ($r^2 = 0.995$) with an overall mean difference of 6.93 g/kg dry matter, and can be described by the equation: ANKOM aNDF (g/kg DM) = 0.9963 x Water Bath aNDF – 4.536. Intercept and slope were not different from zero (P=0.1953) and one (P=0.4828), respectively. Unique intercepts and slopes by sample classification (C_3 grasses, C_4 grasses, legumes, silages) were significant in a multivariate model, but may not be necessary based on the strong overall relationship between ANKOM and WB aNDF. Furthermore, duplicate repeatability for ANKOM and WB was not different. The water bath method is viable for NDF analysis of diverse forage and silage samples, and could provide a low-infrastructure efficient alternative for low-budget laboratories.

1. Introduction

Neutral detergent fiber (NDF) has been adopted worldwide by laboratories, universities, agricultural extension educators and nutritionists as a key indicator of forage nutritive value for use in ration balancing and to support ruminant nutrition research and practitioner decisions. Benefits of NDF over crude fiber (e.g., association with dry matter intake, rumination, fill, passage, and feed intake) are well known (Van Soest, 1994; Mertens, 2003). However, NDF adoption has lagged in many developing countries, particularly in tropical regions where crude fiber is especially poor for prediction of nutritive value. Resistance to NDF adoption has been attributed to the low cost of the crude fiber technique and a large body of proximate analysis-centric ruminant nutrition data in these regions that would become obsolete upon adoption of the detergent system due to poor association between NDF and crude fiber (Van Soest, 1994). Another potential reason is the lack of an accurate, efficient NDF technique with minimal required investment in infrastructure and expertise.

Numerous modifications of the original NDF technique (Van Soest and Wine, 1967) have been suggested (Mascarenhas Ferreira et al., 1983; Van Soest et al., 1991; Giger-Reverdin, 1995; Mertens, 2002). An important modification that enabled rapid processing while reducing technician error was a high throughput NDF technique in filter bags (Komarek, 1994). A newer version of Komarek's original approach consists of a pressurized chamber and more automated processing. Several studies have provided evidence that filter bag techniques correlate well with conventional NDF analysis, often with lower variation, and can be implemented as an acceptable alternative (Komarek, 1993; Vogel et al., 1999; Fay et al., 2005; Ferreira and Mertens, 2007). However, the initial investment in infrastructure can be cost prohibitive, especially for laboratories in developing countries and other low budget operations. Pereira et al. (2009) evaluated an alternative filter bag NDF technique in a shaker water bath. Significant differences were not detected between their method and ANKOM for a limited NDF range of ryegrass, rye, and oats samples.

Development of the technique proposed in this paper was motivated by an observed need in developing countries for an efficient, low-budget NDF analysis alternative using materials that are often readily available in laboratories. Consequently, the primary objectives of this study were: (1) to develop a low-infrastructure filter bag NDF technique, and (2) to test the technique versus a standard filter bag NDF technique (ANKOM) using a diverse set of temperate and tropical forages.

2. Methods

Forage samples (n=196) were collected in New York State and Vietnam between 2010 and 2012 (Table 1), dried to stable weight at 60°C in a forced air oven, and ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen. Samples (0.25 g) were weighed to the nearest 0.0001 g, transferred into filter bags (ANKOM F57, 25 µm porosity), and analyzed separately for aNDF concentration (without sodium sulfite) in duplicate using a standard technique (ANKOM Technology, 2011) and the proposed water bath technique (WB). Sodium sulfite was not added, because samples consisted of forages and silages that did not contain high protein material, material of animal origin, or heat damaged samples, and to eliminate the possibility of sodium sulfite attacking and solubilizing lignin (Van Soest et al., 1991; Hintz et al., 1996). Results were not ash corrected, but were blank bag corrected using the overall blank bag correction factor for each technique. The blank bag correction factor for individual blank bags was calculated as the post-extraction 105ºC dry weight for each bag divided by its pre-extraction weight. The overall blank bag correction factor (BBC) was the mean of individual blank bag correction factors for each technique. Dry matter (DM) was determined for 1 g subsamples dried overnight in a forced air oven at 105ºC, the temperature recommended in National Forage Testing Association Method 2.2.2.5 (Undersander et al., 1993). aNDF concentration was calculated as: g aNDF/kg DM = ((R – BW x BBC)/(SW x SDM)) x 1000, where: R = post-extraction 105ºC dry weight of bag and residue, BW = pre-extraction blank bag weight, BBC = blank bag correction factor for ANKOM or WB, SW = pre-extraction sample weight, and SDM = sample 105ºC DM. A standard *Dactylis glomerata* L. sample was also included in each batch for both techniques to assess variability among batches and techniques for the same sample. Sample processing order was completely randomized. ANKOM and WB batches (48 filter bags or 22 duplicate samples + 2 blank bags + 2 *Dactylis glomerata* L. standards) were completed on the same days using the same neutral detergent solution (NDS) batch. ANKOM guidelines (ANKOM Technology, 2011) for NDS preparation, equivalent to the procedure described by Mertens (2002), were used to prepare 8 L batches. pH tested between 6.9 and 7.1 for all batches. A volume of 4 mL heat-stable alpha-amylase (activity=17,400 Liquefon Unit/ml, FAA, ANKOM Technology) was added for 24 filter bags. Analyses were completed at Cornell University (Ithaca, NY).

Excluding the use of filter bags and batch processing, the ANKOM and WB procedures are closest to the modified procedure for neutral detergent residue (Robertson and Van Soest, 1981) with a modern heat-stable alpha-amylase or the aNDF method (Mertens, 2002) without sodium sulfite.

2.1. Standard filter bag NDF procedure: ANKOM

The ANKOM A200 Fiber Analyzer NDF procedure (ANKOM Technology, 2011) was applied with four modifications: (1) Sodium sulfite was not added. (2) Sample size was 0.25 g instead of 0.5 g. (3) Temperature for post-extraction sample drying was 105ºC instead of 102ºC. (4) Conventional desiccators were used in lieu of desiccator pouches.

Table 1 Forage and silage samples included in the study.

Species	Origin	*n*
C_3 Grasses		**40**
Orchardgrass (*Dactylis glomerata* L.)	New York State	11
Quackgrass (*Elytrigia repens* (L.) Desv. Ex Nevski)	New York State	3
Tall Fescue (*Festuca arundinacea* Schreb.)	New York State	3
Rice straw (*Oryza sativa* L.)	Vietnam	3
Reed Canarygrass (*Phalaris arundinacea* L.)	New York State	11
Timothy (*Phleum pratense* L.)	New York State	9
C_4 Grasses		**122**
Big Bluestem (*Andropogon gerardii* Vitman)	New York State	6
Brachiaria Cv. Mulato II (*Brachiaria ruziziensis* x *B. decumbens* x *B. brizantha*)	Vietnam	75
Guinea Grass (*Panicum maximum* Jacq. (TD 58))	Vietnam	3
Switchgrass (*Panicum virgatum* L.)	New York State	26
Paspalum atratum Swallen	Vietnam	3
Elephant Grass (*Pennisetum purpureum* Schumach.)	Vietnam	5
Maize (*Zea mays* L.)	New York State	4
Legumes		**21**
Birdsfoot Trefoil (*Lotus corniculatus* L.)	New York State	3
Alfalfa (*Medicago sativa* L.)	New York State	14
Stylosanthes guianensis (Aubl.) CIAT 184	Vietnam	1
Red Clover (*Trifolium pratense* L.)	New York State	3
Silages		**13**
Alfalfa silage (*Medicago sativa* L.)	New York State	3
Maize silage (*Zea mays* L.)	New York State	10
Whole Set		**196**

ANKOM samples were processed in the A200 Fiber Analyzer using the new bag suspender design available in 2013 (ANKOM Technology SKU: F11). Two A200 units were used to facilitate parallel batch processing with WB. Each unit contained a vertical cylindrical stainless steel reservoir (2 L capacity) with a stainless steel lid that was closed during the extraction to enable chamber pressurization at 100ºC. Twenty-four filter bags per batch were processed in each unit using the 8-level vertical bag suspender. Each level (tray) was manufactured from heat resistant plastic. Trays were connected by a stainless steel shaft that fit snugly through a center hole in each tray. Each tray contained three filter bags, and each bag was situated in a small pocket where it could float in NDS without contacting other samples during the extraction. After adding ambient temperature NDS (2 L) and alpha-amylase (4 mL), the bag suspender loaded with samples was submerged in the reservoir. A weight was added to the top of the vertical stainless steel shaft to secure the unit and maintain rack submergence during the extraction, and the lid was sealed. Extraction time was 75 min. The A200 units required approximately 15 min to reach target temperature (100ºC), which was maintained for the remaining 60 min of the extraction. Units provided heat and constant vertical agitation by raising and lowering the rack in solution using a mechanical rotating disc. After extraction, heat and agitation were turned off and solution was drained through an exhaust valve at the bottom of the reservoir. The reservoir was refilled with hot water (70 to 90ºC) from a Bunn Model H5E/H5X 18.9 L hot water dispenser (68.1 L boiling water capacity per hour) to complete three rinse cycles (5 min each). Heat and agitation were used during the rinse cycles, but the lid was not sealed. The first two hot water rinses were completed with alpha-amylase (4 mL per rinse). Following the rinse cycles, bags were removed from the tray, gently squeezed to remove excess water, and submerged in acetone (in a glass beaker) for five min. Bags were gently squeezed to remove excess acetone and placed on a glass tray to evaporate remaining acetone. Samples were dried overnight at 105ºC before weighing through conventional desiccators.

2.2. Water bath

The water bath technique replicated the standard procedure (ANKOM) with the following exceptions: (1) Water bath was conducted in a 20 L non-pressurized stainless steel water bath (VWR Scientific Model 1245) fitted with a 2 cm-thick insulating lid. (2) Ten L NDS were used per batch (48 filter bags), 2.5 times the amount of solution needed to immerse bags in the standard procedure. (3) The ratio of heat stable alpha-amylase to filter bags was equivalent to the standard procedure (1 mL per 6 filter bags), but the concentration of amylase to NDS (0.8 mL per L NDS) was lower than the standard procedure (2 ml per L NDS). Forty-eight samples were loaded in a 2-level stainless steel sandwich rack (Figure 1), which was designed to fit the interior dimensions of the water bath. The following materials were used to construct the rack: (1) Four sheets of stainless steel type 304 welded wire cloth (0.2 cm thick wire) with 2.54 x 2.54 cm interior square gaps. The sheets were cut to 44 x 26 cm to fit the interior water bath dimensions (49.5 x 29.2 x 15.2 cm). (2) Four stainless steel type 316 screws (6.35 cm long, 0.635 cm diameter). (3) Flat washers: 32 stainless steel type 316 (3.8 cm outer diameter (OD), 0.635 cm inner diameter, 0.127 to 0.203 cm thick) and 32 with 3.175 cm OD. (4) Twelve stainless steel type 316 thin hex nuts (0.635 cm inner diameter). (5) Ten strips of stainless steel type 304 wire (33 cm long, 0.26 cm diameter). (6) Several packets of 9.7 cm long heat tolerant nylon zip ties. (7) Heat tolerant nylon cord.

Figure 1 A two-level stainless steel sandwich rack was used to process 48 bags per batch.

The rack was assembled and loaded according to the following steps:

(A) Two sheets of welded wire cloth (material 1) were prepared to hold filter bags for each level of the rack. Each sheet was fitted with five strips of wire (material 5) oriented in parallel along the length of the welded wire cloth. The spacing between each wire strip was slightly greater than 5.5 cm to permit filter bag movement and access to solution in the space created between strips (ANKOM F57 filter bag dimensions = 5.0 x 5.5 cm). Each wire strip was attached to the welded wire cloth using three zip ties (material 6) with tie ends clipped and turned to face away from the wire strips to avoid interference with filter bags. Zip ties were added between each wire strip at an interval of two interior square gaps in the welded wire sheet to maintain horizontal separation between filter bags. These zip tie ends were oriented perpendicular to the face of the welded wire cloth and clipped at 2 cm to prevent horizontal movement of filter bags. The wire strips and zip ties created a shallow pocket slightly larger than a single filter bag.

(B) Four screws (material 2) were fitted with a thin hex nut (material 4) tightened down to the head. A 3.175 cm OD flat washer and a 3.8 cm OD flat washer (material 3) were added to the screw with the smaller OD washer facing the screw head. Screws were inserted from below in each corner gap of one sheet of welded wire cloth (from step A). Screw heads faced down and formed the base or "legs" of the rack. The square corner gaps in the welded wire cloth sheet rested on the large OD flat washers. Two additional large OD flat washers were added to the screw after the welded wire sheet. Their combined thickness was approximately equal to the diameter of the wire strips (material 5).

(C) Twenty-four filter bags were loaded in the pockets created by the wire strips and zip ties. Filter bags were oriented to face the center of the rack (Figure 1).

(D) One of the two welded wire sheets not prepared in step A was placed over the filter bags with care to ensure that the zip tie ends remained perpendicular to the sheets and passed through the appropriate gaps in the upper sheet. The sheet rested on the large OD washers added in step B, and on the wire strips. One large and one small OD flat washer were then placed on each screw with the large OD washer facing the rack. The screw and rack were adjusted carefully and a hex nut (material 4) was added to each screw (joint) and tightened to secure the lower level of the rack. A single zip tie was added to further secure each outer long side of the rack to prevent filter bag escape.

(E) Five small OD washers and one large OD washer were added to each screw with the small

OD washers resting on the hex nut (from step D). These washers provided additional spacing for a total of approximately 2.54 cm between the lower and upper levels of the rack. The second welded wire sheet prepared in step A was then added. It rested on the large OD flat washer in each corner. Two additional large OD flat washers were added to each screw after the welded wire sheet. Steps C and D were then repeated to finish preparation of the upper rack level. Protruding wire ends on one side of the welded wire sheets were bent to accommodate a thermostat and thermometer in the water bath.

(F) Four pieces of heat tolerant nylon cord (material 7) were attached to the corners and short side of the loaded rack using two zip ties at each attachment point. Cord purpose was to provide manual rack agitation in the water bath. Cord pieces were fitted for quick attachment and ease in transfer through holes in the insulating lid to permit rack agitation in the water bath without lid removal. The rack loading process required approximately 20 to 30 min per 48 filter bag batch.

VWR Scientific Model 1245 was pre-calibrated at 100ºC with the insulating lid. It required 2.5 h to heat 10 L ambient temperature NDS to 100ºC. The insulating lid was then removed, 8 mL alpha-amylase were added, and the rack was quickly inserted into solution by suspending and lowering it with the nylon cord. Approximately one minute of initial rocking agitation was provided. The four pieces of nylon cord were then passed through pre-made holes in the insulating lid, and the lid was quickly replaced. Four weights were added to secure the lid and a thermometer was inserted through the lid to monitor temperature. The water bath temperature dropped to approximately 90 to 93ºC during the loading process, and required 15 min to reheat to 100ºC. Thus, total time at 100ºC was 60 min of the 75 min extraction, equivalent to ANKOM A200 units. During the extraction, samples were agitated in solution for one of every five min by manually rocking the rack from front-to-back and side-to-side through the insulating lid using the attached nylon cord. The water bath was drained using a 2.54 cm OD flexible PVC tube. Other components of the rinse sequence were identical to ANKOM. Continuous rocking agitation was provided during rinse cycles. The rack unloading process required about five min per rack level. Bag removal, acetone treatment, and drying processes did not differ from ANKOM.

Additional WB details including filter bag rack construction are available in the supplementary video (Supplementary Video 1). During WB development process, numerous iterations were tested with the standard *D. glomerata* L. sample to finalize the procedure before conducting the experiment. Criteria for success were identified (below).

2.3. Duplicate removal criteria

Based on within-laboratory variability in a collaborative study, Mertens (2002) reported that aNDF analyses in beakers should be rerun if duplicate values differ by more than 2.9%, the repeatability or approximate 95% confidence interval for duplicates analyzed in a single laboratory (Mertens, 2003). Lower within-laboratory duplicate variability could be expected using ANKOM filter bag methods based on lower standard deviations or standard errors for most forages and feedstuffs run with ANKOM (Komarek, 1993; Vogel et al., 1999; Fay et al., 2005) versus conventional methods. Thus, ANKOM duplicates and water bath duplicates differing by ≥ 21.0 g/kg were examined to identify erroneous individual aNDF duplicates (poor filtration or open bag issues) based on case-by-case comparison to other duplicate values from both techniques and aNDF concentrations for similar samples. All questionable individual duplicate values were removed. Total number of samples was not impacted, because for each case the remaining duplicate was used in lieu of the duplicate mean for analysis.

2.4. Statistical methods

Simple and multiple linear regressions were undertaken in JMP® Pro 10.0.0 (SAS Institute Inc., Cary, NC) using the standard least squares procedure. A bivariate model of ANKOM aNDF versus WB aNDF was constructed. Multivariate models were also constructed to evaluate additional potential covariates (sample classification category and batch) and interactions. Two-tailed t-tests were used to assess paired mean differences. Mean differences were considered statistically significant when $P<0.05$. Influential samples and outliers were analyzed using Cook's distance measures and outlier box plots, respectively.

3. Results

Eleven total duplicates were removed from the dataset due to probable poor filtration or open bags by applying the 21.0 g/kg duplicate difference criterion explained above. Removed duplicates included: six ANKOM (three *Zea mays* L. silages, one *Medicago sativa* L., and two *Brachiaria* Cv. Mulato II) and five WB duplicates (three *Medicago sativa* L. and two *Zea mays* L. silages). Consequently, pre- and post-removal data for duplicate differences were analyzed separately (Table 2). Post-removal duplicate mean differences and standard deviations were lower than pre-removal levels. Repeatability, a measure of within-laboratory analytical precision based on the variation in results for the same material exposed to similar treatment (e.g., duplicate samples) (AOAC, 2002; Mertens, 2003), was also lower post-duplicate removal. Absolute value of duplicate differences was not significantly different between ANKOM and WB based on a paired *t* test either before or after duplicate removal (Table 2).

Table 2 Pre- and post-adjustment duplicate mean differences and repeatability for ANKOM and water bath (WB).

	Pre-duplicate removal		Post-duplicate removal	
	ANKOM	WB	ANKOM	WB
n	196	196	190	191
Mean[a] (g aNDF/kg DM)	6.35	6.22	5.43	5.43
SD[b] (g aNDF/kg DM)	6.86	6.54	4.20	3.72
SD_r[c] (g aNDF/kg DM))	6.60	6.38	4.85	4.65
Repeatability[d] (g aNDF/kg DM)	18.48	17.85	13.58	13.01
n for mean comparison	196		186[f]	
P[e]	0.8189		0.7892	

[a] Absolute value of mean difference between duplicates.
[b] Standard deviation of the mean difference between duplicates.
[c] Repeatability standard deviation = $(\Sigma d_i^2/2L)^{0.5}$, where d_i is the duplicate difference for sample *i* and *L* is the number of duplicate pairs (AOAC, 2002).
[d] 2.8 x SD_r, the approximate 95% confidence interval for duplicate analyses in the laboratory for this study (AOAC, 2002; Mertens, 2003).
[e] Two-tailed *t* test for paired mean difference between ANKOM and WB duplicate mean differences.
[f] Different duplicates were removed for each procedure. Thus, *n* used in paired *t* test for mean differences was lower than post-duplicate removal *n*.

The overall relationship between ANKOM and WB aNDF was strong and positive (Table 3). Intercept was not different from zero and slope did not differ from unity (Table 3). Samples tended to group by sample classification category (C_3 grasses, C_4 grasses, legumes, and silages) with several exceptions: (1) *Oryza sativa* L. samples had higher aNDF concentrations than other C_3 species, (2) *Z. mays* L. samples grouped more closely with silages and legumes than with other C_4 grasses, and (3) *Stylosanthes guianensis* (Aubl.) CIAT 184 sample grouped with the bulk of C_3 grasses. Numerical differences between ANKOM and WB means broken down by species were low, although some paired mean differences were statistically significant (Table 4).

Table 3 Bivariate ANKOM vs. water bath regression models for the whole dataset and individual sample classes.

Sample Class	*n*	r^2	RMSE (g aNDF/ kg DM)	Slope			Intercept		
				b	SE_b	Prob. *b*=1	*a*	SE_a	Prob. *a*=0
Whole Dataset	196	0.995	10.847	0.9963	0.0052	0.4828	-4.536	3.490	0.1953
C_3 Grasses	40	0.958	8.874	0.8532	0.0290	<0.0001	84.002	17.953	<0.0001
C_4 Grasses	122	0.988	8.151	0.9796	0.0100	0.0438	7.971	7.429	0.2854
Legumes	21	0.939	16.364	1.0532	0.0617	0.3996	-32.796	22.185	0.1557
Silages	13	0.902	11.760	1.0072	0.1002	0.9442	1.697	43.125	0.9693
C_3 Grasses without *Oryza sativa* L. straw	37	0.908	8.220	0.9410	0.0508	0.2533	31.312	30.765	0.3158
C_4 Grasses without *Zea mays* L.	118	0.973	7.882	1.0161	0.0158	0.3077	-19.651	11.842	0.0997
Legumes without *Stylosanthes guianensis* (Aubl.) CIAT 184	20	0.812	16.477	0.9746	0.1105	0.8207	-6.149	38.171	0.8738

Table 4 ANKOM and water bath (WB) descriptive statistics by sample type.

Species	*n*	Mean Difference [b] (%)	P[a]	ANKOM aNDF (g/kg DM)		WB aNDF (g/kg DM)	
				Mean	SD	Mean	SD
Whole Set	**196**	**-1.07**	**<0.0001**	**645.7**	**148.81**	**652.6**	**148.96**
C_3 Grasses	**40**	**-1.08**	**0.0007**	**610.4**	**42.70**	**616.9**	**48.98**
Dactylis glomerata L.	11	0.24	0.5670	610.8	13.19	609.3	18.14
Elytrigia repens (L.) Desv. Ex Nevski	3	-0.78	0.4063	607.7	9.53	612.4	5.68
Festuca arundinacea Schreb.	3	-1.42	0.1256	632.7	15.73	641.6	9.66
Oryza sativa L. straw	3	-4.61	0.0293	726.8	28.06	760.3	19.24
Phalaris arundinacea L.	11	-0.52	0.1626	581.6	32.25	584.7	30.73
Phleum pratense L.	9	-1.93	0.0001	599.7	23.49	611.2	22.17
C_4 Grasses	**122**	**-0.97**	**<0.0001**	**732.4**	**73.07**	**739.5**	**74.13**
Andropogon gerardii Vitman	6	-0.31	0.0802	882.7	7.24	885.4	6.22
Brachiaria Cv. Mulato II	75	-1.26	<0.0001	729.2	23.55	738.4	21.90
Panicum maximum Jacq. (TD 58)	3	-0.32	0.0563	712.1	68.24	714.4	67.30
Panicum virgatum L.	26	-0.71	0.0004	762.5	46.35	768.0	47.33
Paspalum atratum Swallen	3	-1.11	0.2456	698.0	37.53	705.7	36.13
Pennisetum purpureum Schumach.	5	-0.43	0.2183	717.5	35.55	720.6	33.14
Zea mays L.	4	1.46	0.2555	429.5	18.71	423.2	18.67
Legumes	**21**	**-4.09**	**0.0008**	**340.7**	**64.43**	**354.7**	**59.27**
Lotus corniculatus L.	3	-0.62	0.8033	306.3	34.39	308.2	23.03
Medicago sativa L.	14	-6.27	0.0003	326.0	36.66	346.5	33.98
Stylosanthes guianensis (Aubl.) CIAT 184	1	0.91	---	573.7	---	568.5	---
Trifolium pratense L.	3	-0.53	0.4615	366.1	13.73	368.1	16.04
Silages	**13**	**1.10**	**0.1523**	**433.9**	**35.93**	**429.1**	**33.88**
Medicago sativa L. silage	3	0.85	0.2414	444.1	11.58	440.3	11.65
Zea mays L. silage	10	1.18	0.2428	430.9	40.58	425.8	38.03

[a] Two-tailed paired *t* test for mean difference between ANKOM and WB.

[b] Mean difference = ((ANKOM mean – WB mean)/ANKOM mean) x 100.

Multivariate models predicting ANKOM aNDF were tested by adding additional covariates and interactions to the bivariate relationship (ANKOM vs. WB). A processing batch effect was not detected (P=0.2061). For multivariate models including sample classification, the categories were binary (e.g., silage = 1, all other categories = 0) and intercept was the overall intercept. Sample classification was significant (P<0.0001) and is described by the equation: ANKOM aNDF (g/kg DM) = 0.9722 x WB aNDF – 13.244 x legume + 7.531 x silage + 1.413 x C_3 grass + 4.300 x C_4 grass + 9.184. The sample classification by WB aNDF interaction effect was also significant (P=0.0003). Separate bivariate regression equations were generated by sample class to simplify interpretation of this interaction (Table 3). Slopes did not differ from unity except for C_3 grasses and C_4 grasses. The only intercept differing from zero was C_3 grasses.

These outcomes may be strongly impacted by influential samples and outliers, which were evaluated for each sample class using an outlier box plot and Cook's distance measures. Influential samples based on Cook's distance measures greater than 0.4 in decreasing order included the *S. guianensis* (Aubl.) CIAT 184 sample, one *O. sativa* L., one *Z. mays* L., and one *Z. mays* L. silage. Outlier analysis identified the *S. guianensis* (Aubl.) CIAT 184 sample, all *O. sativa* L. samples, two *Z. mays* L. silages (not including the influential sample), and numerous C_4 grasses including: all four *Z. mays* L. samples, one *Panicum maximum* Jacq. (TD 58), all *Andropogon gerardii* Vitman, and four *Panicum virgatum* L. samples. Extreme outliers included all *Z. mays* L. samples, all *O. sativa* L. samples, and the *S. guianensis* (Aubl.) CIAT 184 sample. The extreme outliers group included all influential samples except the silage. This was consistent with species that did not group with others in their sample class (above). Therefore, these species were excluded from their respective sample classes, and the ANKOM – WB bivariate regressions were reevaluated (Table 3). Coefficient of determination decreased in all cases when these species were excluded. Root mean square error (RMSE) decreased for C_3 and C_4 grasses, and increased for legumes. Slopes did not differ from unity and intercepts did not differ from zero. Influential samples were not detected when the same Cook's distance measures cutoff was applied to the full dataset bivariate model with pooled samples classes.

Mean differences were not detected in WB for filter rack level (upper or lower) ($P=0.7822$) or in ANKOM for processing unit ($P=0.6514$). ANKOM blank bag correction factor mean (0.9984 with *SD* ± 0.0015) was higher than WB blank bag correction factor (0.9960 ± 0.0004) ($P<0.0001$, $n=18$). The *D. glomerata* L. standard was higher for ANKOM (603.2 ± 3.6 g aNDF/kg DM) than WB (600.2 ± 3.8 g aNDF/kg DM) ($P=0.0025$, $n=18$), although the numerical difference was low (0.49% of ANKOM standard mean).

4. Discussion

Cost savings of WB over ANKOM consist of the initial investment in ANKOM processing units. Potential WB limitations include processing time, labor, and required NDS. Additional processing time (as compared to ANKOM processing with two A200 units) is required to complete a 48 filter bag aNDF extraction due to delays in heating NDS (dependent on water bath model) and for loading and unloading samples in the stainless steel rack. Labor requirement for WB during extraction is higher than ANKOM, because manual agitation must be provided, an approximate 30 min difference during each extraction and rinse cycle. Rack loading introduces another 20 to 30 min delay for WB. Impact of labor differences could be low in developing country laboratories.

Time differential noted above could be mitigated or shifted in favor of WB by adding additional vertical levels to the stainless steel rack. Our rack contained two levels with 24 filter bags each (Figure 1) to facilitate parallel processing with two ANKOM A200 machines. Space remained in the WB rack for four additional bags on each level (two on either side). VWR Scientific Model 1245 water bath depth would easily accommodate four vertical levels, thus permitting up to 112 filter bags per batch processed with approximately 16 to 18 L NDS (1.7 to 2 times the amount of NDS required by ANKOM).

Chemical costs could become a limitation of the technique for high throughput laboratories. Other water bath or rack designs may permit more economical use of NDS. Pereira et al. (2009) used a metallic basket to submerge 60 samples per batch in a shaker water bath at 99ºC. No significant differences between their method and ANKOM were detected for ryegrass, rye, or oats samples (overall ANKOM mean = 313.3 with *SE* ± 3.8 g NDF/kg DM). However, samples with greater filtration issues (e.g., corn silages) could be problematic with many filter bags submerged loosely en masse in a metal basket.

Collection and reuse of NDS may be a possibility, especially for NDS from batches with high mean NDF concentration. In an oven NDF technique test, Chai and Udén (1998) reported that NDS as low as 25% of the strength recommended by Van Soest et al. (1991) would be adequate for most sample classes. Recycling expensive reagents such as sodium lauryl sulfate, ethylenediaminetetraacetic acid, and acetone could further economize NDF analyses (Van Soest, 2015), possibly offsetting differences in NDS volume requirements.

Modifications to the ANKOM NDF procedure described here should have no negative impact on overall results. Sodium sulfite was not added (reasons described above). Sample size has been implicated as an important factor in conventional NDF analyses (Cherney et al., 1985). Sample size reduction from 0.5 to 0.25g was implemented to improve filtration by decreasing potential for within-bag sample agglomeration, particularly with starch-containing samples (Cherney et al., 1989). Neutral detergent fiber has been determined successfully using very small sample size, from 10 to 50 mg (Pell and Schofield, 1993). After removal from acetone, samples were dried at 105ºC, instead of the 102ºC indicated in the ANKOM procedure, because lower average bias was reported at this temperature using National Forage Testing Association Method 2.2.2.5 compared to other common DM methods (Thiex, 2008).

Repeatability of duplicates for ANKOM and WB (Table 2) was numerically lower than aNDF within-laboratory repeatability in a collaborative study (Mertens, 2002). This difference can probably be attributed to batch processing with filter bags, which helps reduce technician error and provides more consistent treatment of duplicate samples. Furthermore, repeatability was numerically lower for WB than ANKOM (Table 2), suggesting that in our laboratory with a single equipment operator, WB precision did not differ from ANKOM precision. Operator conscientiousness is important to achieving acceptable precision with WB, because the technique is less automated than ANKOM.

Numerical magnitude of aNDF mean differences between ANKOM and WB was generally low (Table 4), and potentially inconsequential relative to other sources of variation in gravimetric techniques (e.g., within sample variation, DM measurement, weighing inconsistencies, desiccator use, and sample size) (Mertens, 2003). For most purposes, WB sample deviations of ± 20 g aNDF/kg DM from the standard technique would fall within acceptable levels (Table 4). Only 11 of 196 individual samples (5.6%) exceeded this threshold. However, certain sample types exhibited significant aNDF mean differences between techniques (Table 4). For example, *M. sativa* L. and *O. sativa* L. WB means were higher than ANKOM (Table 4), and the overall bivariate regression equation (Table 3) slightly overestimated ANKOM aNDF concentration for these samples. In these cases, pressurization and agitation differences between techniques may affect filtration. There was also a significant mean difference between ANKOM and WB for *D. glomerata* L. standards, but it was numerically low and consistent with other *D. glomerata* L. samples in direction and magnitude of deviation from ANKOM (Table 4).

The impact of slightly greater deviation from ANKOM for some sample types would depend on desired laboratory accuracy. Goering and Van Soest (1970) reported that samples containing high “starch, protein, or other mucilaginous materials” are more likely to cause NDF filtration issues. Starch content in *M. sativa* L. and *O. sativa* L. samples was probably low, and unlikely to be associated with observed differences since mean differences were not detected for samples likely to be high in starch (e.g., *Z. mays* L. and *Z. mays* L. silages) (Table 4). Protein content in *M. sativa* L. was probably higher than most other species analyzed, and may have contributed to higher aNDF values in WB. However, a mean technique difference was not observed for *M. sativa* L. silages. *O. sativa* L. straw samples were probably high in NDF and silica, and low in protein (Van Soest, 2006). Relative to other straws, they would also be low in lignin. These typical characteristics of *O. sativa* L. straw should not impact filtration. Thus, possible reasons behind the ANKOM – WB aNDF discrepancy for these samples are not known. Mean differences for these species were still below 7% of the ANKOM aNDF mean. The impact on overall WB aNDF ranking of these samples compared to other similar samples would be minimal. Thus, even for species with greater differences between ANKOM and WB aNDF values, the practical significance may be low.

The statistical significance of the sample classification effect and its interaction with WB aNDF in a multivariate model implicated alternative regression intercepts and slopes based on sample classification. Maximum difference from the overall intercept in a model without the interaction effect was for legumes (– 13.244 g aNDF/kg DM). Interaction assessment via bivariate ANKOM – WB models by sample class indicated the greatest slope difference from the whole dataset regression for the C_3 grass model (Table 3). C_3 grasses were the only sample class in which slope and intercept differed from unity and zero, respectively. Significant slope and intercept differences were no longer detected after exclusion of extreme outliers and influential samples (all *Z. mays* L., all *O. sativa* L., and the *S. guianensis* (Aubl.) CIAT 184). Notably, *Z. mays* L.

samples (C_4) were grown in a temperate environment, *O. sativa* L. samples (C_3) were grown in a tropical environment, and the *S. guianensis* (Aubl.) CIAT 184 sample was the only legume grown in a tropical environment. Thus, sample class by environment interactions may have contributed to these species not grouping well with others in their class. Furthermore, based on strength of the overall WB – ANKOM relationship (Table 3, whole dataset) and the lack of influential samples, unique intercepts and slopes by sample classification may not be necessary.

Several additional criteria were identified for WB success. First, the water bath unit must deliver uniform 100ºC heat throughout the solution in order to achieve consistent within and across-batch aNDF results. Samples should also be exposed to 100ºC temperatures for the same amount of time as A200 units (1 h). Water bath calibration using an insulating lid is necessary for accurate temperature control. Lid removal should be avoided during the extraction to prevent rapid heat loss and temperature fluctuations. Filter bag rack materials must be robust and not interact with boiling NDS. Stainless steel (type 304 or 316) is suggested, although heat-resistant plastic could be considered (polyketone-type plastic). Typical plastics (e.g., polyvinyl chloride) are not viable, because they reach the glass transition point below boiling. Regardless of rack material, design should provide individual filter bags with consistent separation and ample NDS access to improve the odds of successful filtration.

5. Conclusions

The water bath filter bag technique proposed in this paper generated aNDF concentrations and repeatability that were consistent with the ANKOM Technology standard method for a variety of forages and silages. The method could be a viable alternative to ANKOM for low-budget laboratories.

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