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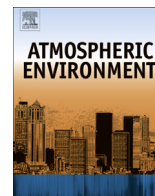
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## Review

Emission of volatile organic compounds from silage: Compounds, sources, and implications<sup>☆</sup>Sasha D. Hafner<sup>a,\*</sup>, Cody Howard<sup>b</sup>, Richard E. Muck<sup>c</sup>, Roberta B. Franco<sup>d</sup>, Felipe Montes<sup>a,1</sup>, Peter G. Green<sup>e</sup>, Frank Mitloehner<sup>d</sup>, Steven L. Trabue<sup>f</sup>, C. Alan Rotz<sup>a</sup><sup>a</sup> USDA Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, 3702 Curtin Rd., University Park, PA 16802, USA<sup>b</sup> US Environmental Protection Agency, Office of Research and Development, National Center for Environmental Research, 1200 Pennsylvania Ave. N.W., Washington, DC 20460, USA<sup>c</sup> USDA Agricultural Research Service, US Dairy Forage Research Center, 1925 Linden Drive West, Madison, WI 53706, USA<sup>d</sup> Department of Animal Science, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA<sup>e</sup> Department of Civil and Environmental Engineering, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA<sup>f</sup> USDA Agricultural Research Service, Soil, Water, and Air Resources Research Unit, 2110 University Blvd. Ames, IA 50011, USA

## H I G H L I G H T S

- Silage emits volatile organic compounds (VOCs) that contribute to poor air quality.
- In this review, we identified the most important compounds and their sources.
- Alcohols make the largest contribution to VOC emissions and ozone formation.
- Bacteria and yeast probably produce most silage VOCs during fermentation and storage.
- Management practices, in particular silage additives, may help reduce VOC emission.

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## A B S T R A C T

Silage, fermented cattle feed, has recently been identified as a significant source of volatile organic compounds (VOCs) to the atmosphere. A small number of studies have measured VOC emission from silage, but not enough is known about the processes involved to accurately quantify emission rates and identify practices that could reduce emissions. Through a literature review, we have focused on identifying the most important compounds emitted from corn silage (the most common type of silage in the US) and the sources of these compounds by quantifying their production and emission potential in silage and describing production pathways.

We reviewed measurements of VOC emission from silage and assessed the importance of individual silage VOCs through a quantitative analysis of VOC concentrations within silage. Measurements of VOC emission from silage and VOCs present within silage indicated that alcohols generally make the largest contribution to emission from corn silage, in terms of mass emitted and potential ozone formation. Ethanol is the dominant alcohol in corn silage; excluding acids, it makes up more than half of the mean mass of VOCs present. Acids, primarily acetic acid, may be important when emission is high and all VOCs are nearly depleted by emission. Aldehydes and esters, which are more volatile than acids and alcohols, are important when exposure is short, limiting emission of more abundant but less volatile compounds.

Variability in silage VOC concentrations is very high; for most alcohols and acids, tolerance intervals indicate that 25% of silages have concentrations a factor of two away from median values, and possibly much further. This observation suggests that management practices can significantly influence VOC concentrations. Variability also makes prediction of emissions difficult.

The most important acids, alcohols, and aldehydes present in silage are probably produced by bacteria (and, in the case of ethanol, yeasts) during fermentation and storage of silage. Aldehydes may also be

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produced aerobically by spoilage microorganisms through the oxidation of alcohols. Abiotic reactions may be important for production of methanol and esters. Although silage additives appear to affect VOC production in individual studies, bacterial inoculants have not shown a consistent effect on ethanol, and effects on other VOCs have not been studied. Production of acetic acid is understood, and production could be minimized, but a decrease could lead to an increase in other, more volatile and more reactive, VOCs. Chemical additives designed for controlling yeasts and undesirable bacteria show promise for reducing ethanol production in corn silage.

More work is needed to understand silage VOC production and emission from silage, including: additional measurements of VOC concentrations or production in silage of all types, and an exploration of the causes of variability; accurate on-farm measurements of VOC emission, including an assessment of the importance of individual ensiling stages and practices that could reduce emission of existing VOCs; and work on understanding the sources of silage VOCs and possible approaches for reducing production.

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## 1. Introduction

The vast majority of agriculture air quality research from animal feeding operations has focused on odor, ammonia, or particulate material emissions. However, emissions of volatile organic compounds (VOCs) from silage, a common type of fermented cattle feed, has been identified as a leading contributor to poor air quality in the San Joaquin Valley in central California (Howard et al., 2010). Silage is produced by storing chopped forage (most commonly whole-crop corn in the US, but also grass, alfalfa, and other crops) under anaerobic conditions to promote bacterial fermentation and, ultimately, preservation (Wilkinson, 2005). It is a major component of the diets of dairy and beef cattle in the US and elsewhere. Emission measurements have shown that silage emits numerous VOCs (more than 50) that can contribute to ozone formation in the troposphere (Chung et al., 2009; Howard et al., 2010; Hu et al., 2012). Interest in the contribution of silage emissions has focused on the San Joaquin Valley in central California, US, a largely rural area with a high concentration of large dairy farms and persistent air quality problems (Alanis et al., 2008; Pusede and Cohen, 2012). However, considering the widespread use of silage, it probably affects air quality in other regions, particularly those with meteorological conditions that contribute to ozone formation.

Although the role of VOCs in the production of ground-level ozone has been studied for many years, interest in the effect of silage VOC emissions on air quality is a recent development, and there is a lack of available data on VOC production and emission from silage. At this time, it is not possible to make reliable estimates of the magnitude of VOC emissions from farms, or to identify management practices that can reduce it, since little is known about the mass of VOCs that is typically present in silage, and potentially available for emission. Progress on the problem of silage and air quality will require assessment of the relative effect of VOC groups or even individual compounds on air quality, among other research needs.

The present work was carried out to address some of these limitations. In particular, our objectives were to: 1) identify those silage VOCs that make the largest contributions to VOC emissions and ozone formation, 2) describe the pathways through which these compounds are produced in silage, and, 3) identify research needed to develop accurate emission estimates and identify management practices that will reduce emission.

We used two approaches to assess the importance of individual silage VOCs: a review of measurements of VOC emission from silage, and a compilation of the concentrations of VOCs present within silage. While both sources of data have limitations, they are the best available sources on silage VOCs. Emission measurements provide a direct estimate of the magnitude of emissions from silage and mixed cattle feed, however, measurement techniques may not

be accurate, and only five studies have directly measured emission rates (Table 1). More data are available through the measurement of VOCs present within silage. Since VOC emission is related to concentrations within silage (Hafner et al., 2012), concentration data can provide an indirect indication of the magnitude of emissions. We developed quantitative indices to assess the importance of individual compounds (i.e., their relative contribution to emissions and ozone formation) under possible emission scenarios.

We also reviewed information on VOC production in silage. Work has been carried out on understanding the production of some fermentation end products in silage, in particular, those compounds that affect the preservation of silage: lactic and acetic acids. Less is known about other compounds.

## 2. Data sources and methods

Interest in VOC emission from silage is relatively new, and measurements of emission rates have only recently been reported. We summarized and discussed the significant results from studies that reported measurements of VOC emission from silage (Table 1), with a focus on identifying those compounds that make the largest contribution to VOC emissions or ozone formation.

Most recent studies on emission of silage VOCs have sought to identify and quantify the major VOC components of silage emissions through field or laboratory measurements (Alanis et al., 2008;

**Table 1**  
Studies that report VOC emission measurements from silage.

Study <sup>a</sup>	Year	Silage sources	Method	VOC group <sup>b</sup>					
				A	B	C	D	E	F
1	2008	Corn silage pile and mixed feed	Flux chamber	6					
2	2009	Corn silage pile and mixed feed	Flux chamber	2	1		6	42	
3	2010	Corn, alfalfa, and small grain silage and mixed feed	Environmental chamber <sup>c</sup>	13		8	22	39	
4	2010	Corn silage	Wind tunnel	1					
5	2010	Corn silage	Wind tunnel and flux chamber	1					
6	2011	Corn, alfalfa, and small grain silage and mixed feed	Headspace method and environmental chamber	9	15		7	35	
7	2012	Corn silage	Mass balance	1					

<sup>a</sup> Studies: 1. Alanis et al. (2008), 2. Chung et al. (2009), 3. Howard et al. (2010), 4. Montes et al. (2010), 5. Hafner et al. (2010), 6. Malkina et al. (2011), 7. Hafner et al. (2012).

<sup>b</sup> Groups: A = acids, B = alcohols, C = esters, D = aldehydes, E = ketones, and F = other compounds. Values in table are the number of compounds in each group.

<sup>c</sup> In addition to emission measurement, ozone formation was studied using a smog chamber and a model.

Chung et al., 2009; Howard et al., 2010; Montes et al., 2010; Hafner et al., 2010; Malkina et al., 2011; Hafner et al., 2012), while two studies have looked at ozone formation through computer simulations (Howard et al., 2010; Hu et al., 2012). While there is no recommended standard method for measuring VOC emission from silage, most studies have followed the same basic principle for both field and laboratory measurements. The “emission isolation flux chamber” (Kienbusch, 1986) or a similar device has been used in two studies, and a small wind tunnel and a room-sized environmental chamber have also been used (Table 1). In all of these approaches, gas-phase VOC concentrations or masses were measured over time and combined with air flow measurements to calculate cumulative emission or emission rate.

Air flow conditions near the silage surface have been shown to influence VOC emission rates (Montes et al., 2010; Hafner et al., 2010), and all of the approaches listed above suffer from the difficulty of replicating air flow conditions that animal feed is exposed to on farms. Additionally, individual point measurements of flux reported in these studies do not capture changes that occur over time as VOCs in the source are depleted. The studies that used flux chambers or environmental chambers measured emission rates after short exposure times: 30 min (Alanis et al., 2008; Chung et al., 2009), 6 min (Howard et al., 2010), and 10 min (Malkina et al., 2011). Hafner et al. (2012) used a mass balance technique to estimate cumulative emission, which may eliminate these problems, but produces fewer data. Estimates of emission rates should be treated as approximate and relative at best.

To describe the pathways through which silage VOCs are produced, we summarized information on VOC production from other studies. Because the topic of silage VOC emission has only recently received attention, the production of some important compounds in silage has not been studied. However, potential pathways for producing all important groups of silage VOCs have been described in the literature.

To quantitatively assess the importance of individual VOCs present in silage, we compiled data on silage VOC concentrations, volatility, and reactivity. Emission of VOCs from silage could occur during each stage of silage production, including harvest and transport, packing and sealing, fermentation, storage, feedout (when a silage pile is open to the atmosphere), and feeding. Concentrations of VOCs present in silage provide an indication of total quantities produced, and therefore available for emission. Furthermore, emission rates are related to concentrations, even when only a small fraction the available mass is lost (Hafner et al., 2012). Measurement of VOCs within silage took place after fermentation and storage for the data we compiled. Losses that occur before measurement takes place and production that occurs after would not be reflected in these concentration measurements. Because silage should not be exposed directly to the atmosphere until the end of storage, when feeding occurs, it seems unlikely that significant emission occurs during fermentation and storage before the silo is opened (although preliminary calculations suggest that this may not be true for esters and aldehydes, which are among the more volatile compounds present.)

For most compounds, it is likely that the most significant VOC production occurs during fermentation and storage of silage and not during exposure to air. But living and recently harvested plants also emit VOCs, and emission is stimulated by mechanical damage such as cutting (Holopainen, 2004). Total VOC mass emitted during cutting (and in some cases, drying for hay production) grass, clover, or alfalfa have been measured to range from about 300 to 700 mg kg<sup>-1</sup> (per kg of harvested dry matter) (Karl et al., 2001; Warneke et al., 2002; Davison et al., 2008). In these and other studies (de Gouw et al., 2000; Graus et al., 2013), methanol has been found to be the dominant compound. Based on the data

discussed below, the total quantity of VOCs present in finished silage is much higher than these losses. More work is clearly needed to quantify emission before and after ensiling, and losses during crop harvest and wilting are beyond the scope of this study.

Even with the limitations discussed in the preceding paragraphs, data on VOC concentrations within silage collected from within a silo or silage pile should provide a reasonable estimate of VOC production in silage, and the types and quantities of VOCs available for emission. But more work is clearly needed on assessing the importance of each silage production stage for VOC emission.

We found more than 30 peer-reviewed papers that reported concentrations of non-acid VOCs within corn and, to a lesser extent, grass silage (Table 2). We found very few data for other types of silage, and so focused on corn silage, which accounts for around

**Table 2**  
Data sources for silage VOC concentrations.

Study <sup>a</sup>	Pub. year	Crop <sup>b</sup>	VOC group <sup>c</sup>					Number of		
			A	B	C	D	E	Obs. <sup>d</sup>	Silages <sup>e</sup>	
1	1974	c, g, l		4			1		49	10
2	1982	c		1					1	1
3	1987	c, g, l, sg	4						21	320
4	1987	c, g, l, sg		5					40	320
5	1989	c, g	3	2		5	2		24	37
6	1999	c		2					18	9
7	2000	g		1					156	78
8	2003	c		2					12	6
9	2004	c		1					4	4
10	2004	c		1					6	6
11	2004	c		1					8	8
12	2004	c, g	1	1					5	552
13	2005	g		2					24	12
14	2006	c		1			1		15	8
15	2006	c	2	1					9	162
16	2007	g	3	4	13	5			25	24
17	2007	c	4	4	1				29	46
18	2009	c, g					8		16	32
19	2009	c		1					6	6
20	2009	c, g, l, sg	3						41	62,376
21	2009	c		1					3	3
22	2009	c		13	5				34	2
23	2010	c		1					3	3
24	2010	c		3	1				36	117
25	2010	c		3	2	1			30	5
26	2010	c		1					4	4
27	2010	c		1					15	15
28	2011	c		1					5	5
29	2011	c		2					24	12
30	2011	c, sg		1					4	4
31	2012	c, g, l	3	4	5				97	8880
32	2012	c, g, sg	1						4	13,346
33	2012	c, g, l	4						12	54,312
34	2012	c		3			1		16	4
35	2012	c		1					10	10

<sup>a</sup> Studies: 1. Hartman (1974), 2. Byers et al. (1982), 3. Kalac (1987), 4. Kalac and Pivnickova (1987), 5. Langin et al. (1989), 6. Driehuis et al. (1999), 7. Driehuis and van Wikselaar (2000), 8. Nishino et al. (2003), 9. Filya (2004), 10. Kung et al. (2004), 11. Rodrigues et al. (2004), 12. Sorensen (2004), 13. Nishino and Touno (2005), 14. Kim and Adesogan (2006), 15. Kleinschmit and Kung (2006), 16. Krizsan et al. (2007), 17. Nielsen et al. (2007), 18. Chmelova et al. (2009), 19. Huisden et al. (2009) 20. Rock River Laboratory (2009), 21. Tabacco et al. (2009), 22. Weiss (2009), 23. Hafner et al. (2010), 24. Kristensen et al. (2010), 25. Raun and Kristensen (2010), 26. Reich and Kung (2010), 27. Schmidt and Kung (2010), 28. Contreras-Govea et al. (2011), 29. Li and Nishino (2011), 30. Addah et al. (2011), 31. Cumberland Valley Analytical Services (2012), 32. Dairyland Laboratories (2012), 33. Dairy One (2012), 34. Hafner et al. (2012), 35. Teller et al. (2012).

<sup>b</sup> Crops: c = corn, g = grass, l = legume, sg = small grain (includes wheat, oats, barley, rye, and other small grains).

<sup>c</sup> Groups: A = acids, B = alcohols, C = esters, D = aldehydes, E = ketones, and F = other compounds. Values in table are the number of compounds in each group.

<sup>d</sup> Total number of observations used in current study (one observation = single value for a single compound).

<sup>e</sup> Total number of silages analyzed in study.

85% of the silage used in the US (Wilkinson and Toivonen, 2003). Mean concentrations, expressed on a dry matter basis, were taken from the papers. Where results from multiple treatments were reported, means from all treatments were included separately. One study (Weiss, 2009) reported only minima and maxima, and we used the mean of these values in our analysis. Several studies reported observations that were below detection limits, although most did not provide detection limits. We set all values below detection limits to  $5 \text{ mg kg}^{-1}$ , and excluded from our analysis compounds without at least one observation above  $10 \text{ mg kg}^{-1}$  (all on a dry matter basis). We also excluded three compounds with very low volatility: lactic acid, 1,2-propanediol, and propylene glycol. Acid data were taken from a few large forage laboratory compilations and also from those studies reporting data on other VOCs which included measurements for more than 10 silages.

Data on alcohol and acid concentrations in individual silage samples were supplied by two forage laboratories in the US: Rock River Laboratory (2009) and Cumberland Valley Analytical Services (2012). Cumberland Valley Analytical Services also provided ester concentrations for individual silages. For all of these data, we calculated mean values by crop, and, if possible, agricultural region (United States Department of Agriculture, 1998). Lastly, we included mean VFA concentrations by crop from online summaries posted by two forage laboratories (Dairyland Laboratories, 2012; Dairy One, 2012).

To quantify the contribution of each compound to emissions and ozone formation, we developed indices that reflect emission mass and potential ozone formation. The contribution of a particular compound emitted from a farm to ozone formation within its airshed is dependent on the quantity of the compound emitted, and the tendency of the compound to form ozone (its reactivity,  $r$ ). The mass of a compound emitted from a particular silage pile is related to its equilibrium gas-phase concentration within silage, which is proportional to the concentration of the compound within silage (all else being constant). The equilibrium gas-phase concentration is also approximately proportional to a compound's volatility, as quantified by the Henry's law constant. These relationships have been quantitatively described (Hafner et al., 2012).

Maximum incremental reactivity (MIR) and equal benefit incremental reactivity (EBIR) are measures of the potential for VOCs to contribute to ozone formation under defined conditions, and are often used to quantify VOC reactivity in the atmosphere (Carter, 2009). For the present work, we used EBIR to quantitatively describe reactivity, because it is based on conditions that more closely resemble those in rural areas than is MIR (Howard et al., 2010). The actual contribution of some mass of a particular compound within the atmosphere to ozone formation depends on chemical and physical conditions in the atmosphere, and it is likely that even EBIR overestimates ozone produced from silage VOCs in central CA (Hu et al., 2012). We used EBIR only as a relative measure of reactivity here. Following these principles, we developed four indices to quantify the relative importance of each VOC:

1.  $I_1 = c$ , where  $c$  = concentration. Reflects the total mass of each compound available for emission.
2.  $I_2 = Hc$ , where  $H$  = Henry's law constant (gas:aqueous). Reflects the emission rate when the mass of VOC present in silage is not limiting emission.
3.  $I_3 = rc$ , where  $r$  = reactivity. Reflects the total mass of ozone that each compound could produce if it were all emitted.
4.  $I_4 = Hrc$ . Reflects the potential ozone production rate when the mass of VOC present within silage is not limiting emission.

No single index accurately quantifies relative contributions to emissions and ozone formation for all compounds and times, but

the initial emission rate just after exposure is approximately proportional to  $I_2$ , and cumulative emission after all VOCs have volatilized is proportional to  $I_1$ . For example, when a fresh layer of silage is first spread in a thin layer, initial emission rates would be proportional to  $I_2$ , and ozone production would be proportional to  $I_4$ . After a few hours  $I_2$  and  $I_4$  would overpredict emission rates and ozone production for those most volatile compounds that have been depleted. If the sample were left exposed to the atmosphere until all compounds were depleted, cumulative emission and ozone production would be proportional to  $I_1$  and  $I_3$ , respectively.

We calculated values of indices  $I_1$  through  $I_4$  for all observations. Reactivity values were taken from Carter (2009), but were not available for ten compounds. In these cases, reactivities were set to values for similar compounds (e.g., values for 3-methylbutanal were used for 2-methylbutanal) or values were extrapolated by linear regression from smaller analogous compounds (e.g., values for ethyl valerate were extrapolated using regression results for reactivity vs. the number of carbon atoms for ethyl formate, ethyl acetate, ethyl propionate, and ethyl butyrate).

For Henry's law constant, we used the median of measured values at  $25^\circ\text{C}$  from Sander (2011) when measured values were available. For those compounds without measurements, we used predicted values from ChemSpider (<http://www.chemspider.com/>). Henry's law constants were converted to unitless values (gas:aqueous) based on the ideal gas law.

We calculated mean values of all four indices for each compound in two steps. First, a geometric mean was calculated for each study, ignoring any differences in treatments or other conditions. These means were then used to calculate an overall geometric mean, which was weighted by the number of individual silages in each study. Indices  $I_2$  and  $I_4$  do not have meaningful units, and although they could be converted to an equilibrium gas-phase concentration, for example, we normalized them so that the sum of mean values was 1.0 to facilitate evaluating the importance of individual compounds or groups.

### 3. Results and discussion

#### 3.1. Measurements of VOC emission from silage

##### 3.1.1. Field-based measurements

Alanis et al. (2008) measured emission of volatile organic acids from the California State University Fresno Dairy. Emission rates were measured from the uncovered face of a corn silage pile, mixed feed (primarily corn silage) fed to cattle within the barn, and non-feed sources using a flux chamber. These researchers found that six acids were emitted from silage: acetic acid, butanoic acid, hexanoic acid, pentanoic acid, propanoic acid, and 3-methyl butanoic acid (in descending order of importance). Acetic acid accounted for 70–90% of the total acid emissions from silage and mixed feed, and these two sources were estimated to be the greatest sources of acid emissions on the farm. The mean flux of acetic acid from silage was about  $1.5 \text{ g m}^{-2} \text{ h}^{-1}$ .

Emission of VOCs was measured by Chung et al. (2009) at six separate dairies located in California's Central valley. At each location, measurements were made for a corn silage pile, mixed feed (containing corn silage) fed to cattle, and other non-feed sources, also using a flux chamber. Acids were not measured. Forty-eight compounds were identified. The silage pile and the mixed feed in the feed lanes represented the greatest source of emissions identified among the six sources on each dairy farm. Ethanol was the dominant compound measured from both mixed feed and silage, with a flux between  $0.2$  and  $0.3 \text{ g m}^{-2} \text{ h}^{-1}$ . Other important compounds emitted from these sources included ethyl acetate, acetone, and 2-propanol. Alkanes, aromatic compounds, alkenes, and

halogenated organics were also emitted from cattle feed, but at rates orders of magnitude lower than ethanol.

### 3.1.2. Laboratory-based measurements

Three laboratory studies on VOC emission have been published (Montes et al., 2010; Hafner et al., 2010; Malkina et al., 2011). Malkina et al. (2011) measured VOC emission from silage and other feed samples from a commercial dairy using a room-sized environmental chamber at the University of California, Davis. In addition, these researchers screened for VOCs using a headspace technique, to identify compounds (but not quantify emission) from the samples.

Using the headspace method, Malkina et al. (2011) identified 24 compounds which were emitted from corn, alfalfa, and cereal silage and mixed feed. Alcohols made up the largest fraction of the total VOC emissions and ethanol was the dominant VOC emitted from silage. Other compounds identified included five acids and thirteen esters. The results from their emission measurements showed that ethanol was the most abundant VOC, representing 70% of the total alcohols emitted, where alcohols accounted for >80% of the total mass of VOCs emitted. Other important compounds included 1-propanol and unidentified C<sub>3</sub> and C<sub>4</sub> alcohols. Acetaldehyde was identified as the most abundant carbonyl compound.

Montes et al. (2010) and Hafner et al. (2010) used a wind tunnel system to measure emission of a single compound, ethanol, from packed and loose corn silage over 12 h. Packed silage was collected from a bunker silo without disturbing the structure of the sample. Emission rate was found to be dependent on exposure time, air velocity, temperature, and silage porosity. These studies measured the total ethanol content in silage, so emission could be related to the total mass present. Ethanol emission from three types of packed and loose corn silage samples (15 cm deep) exposed to a range of temperatures (5°C–35°C) and air velocities (0.05–5 m s<sup>-1</sup>) in a wind tunnel ranged from 1% to 80% of the initial mass over 6 h, with emission increasing with temperature and air velocity (Montes et al., 2010; Hafner et al., 2010). Under intermediate conditions (20°C, air velocity of 0.5 m s<sup>-1</sup>), ethanol loss from packed silage ranged from 3 to 13% over 6 h, and 5–20% over 12 h, with much of the variation explained by gas-phase porosity. Two types of loose silage that differed in particle size lost ethanol at a higher rate: 13%–51% over 6 h and 20%–77% over 12 h at 20°C and 0.5 m s<sup>-1</sup>. Instantaneous flux ranged from below 1 g m<sup>-2</sup> h<sup>-1</sup> to more than 100 g m<sup>-2</sup> h<sup>-1</sup>, depending on conditions and exposure time. Exposed corn silage particles lost ethanol much more rapidly—about 50% of the initial mass within the first 5 min (Hafner et al., 2010).

Hafner et al. (2012) used a mass balance approach for measuring VOC emission without the biases that can accompany flux chamber and wind tunnel measurements. Loose corn silage samples (10 cm deep) exposed to air flow within a barn and natural wind outside emitted 21%–60% of the initial mass of alcohols (methanol, ethanol, and 1-propanol) over 6 h. The loss of acetaldehyde in these trials ranged from 45% to 92% of the initial mass, and was always greater than the loss of alcohols.

### 3.1.3. Model analysis

Using a transportable smog chamber, Howard et al. (2010) measured VOC emission and determined the ozone formation potential of a variety of feeds including corn silage and mixed feed. Emission of VOCs was measured in a room-sized environmental chamber. Once the chemical components of the silage VOC emissions had been identified, they were lumped into chemical categories for model analysis. These data were used as input for a chemical reaction model to predict the total ozone production potential of feed emissions and individual VOC groups. The

chemistry mechanism and lumping scheme used in this work are described in an earlier paper (Howard et al., 2008).

Emission measurements showed that ethanol and higher weight alcohols were the most abundantly emitted VOCs from corn silage, alfalfa silage, and mixed feed, while acetaldehyde, alkenes, and ketones were also measured in significant quantities. For cereal silage, ethanol was by far the most abundant compound, but large amounts of acetaldehyde and higher weight alcohols were emitted as well. Model analysis found that higher weight alcohols accounted for over half the ozone formation in corn silage, with alkenes and ethanol accounting for the remainder. For alfalfa silage, higher weight alcohols accounted for nearly 85% of the ozone formation and alkenes and ethanol attributed the remaining amount. In cereal silage emissions, aldehydes, ethanol, and higher weight alcohols each accounted for roughly a third of the ozone formation. Finally, higher weight alcohols and aldehydes contributed most of the ozone formation in mixed feed, with ethanol and alkenes accounting for the remaining portion.

In a later publication (Hu et al., 2012), these emission data were used to compare the potential impact livestock feed and mobile sources had on regional ozone formation in Central California through simulation modeling. The simulation indicated that silage emissions contribute as much as 30% of the ground level peak O<sub>3</sub> (8-h average) in Central California compared to mobile sources. However, as emissions from mobile sources decrease in the coming years, the relative importance of silage was predicted to increase, and the two were predicted to be similar around 2020.

## 3.2. VOCs within silage

Forty-six VOCs have been measured in silage, and of these, 32 have been measured at concentrations above 10 mg kg<sup>-1</sup> (Table 3). Most of these VOCs are acids, alcohols, esters, or aldehydes; the only exceptions are 3-hydroxy-2-butanone (acetoin) and two other ketones. The VOC with the highest concentration in corn silage is acetic acid (overall mean of 18 g kg<sup>-1</sup>), and other acids are generally much lower (Fig. 1). Ethanol is the next most concentrated VOC (overall mean of 7.7 g kg<sup>-1</sup>), with the concentrations of some observations overlapping those of acetic acid. Aldehydes, esters, and ketones are present at much lower concentrations; only two compounds had overall mean concentrations above 500 mg kg<sup>-1</sup>. Importantly, VOC concentrations are highly variable. Ethanol, for example, varied by more than two orders of magnitude (120-fold) in our compilation, while propanol varied by more than three orders of magnitude (2800-fold). This variability carries through for all the indices discussed in this section, and has implications for predicting and controlling VOC emission from silage.

When both volatility and concentration are considered (as in index I<sub>2</sub>) the relative importance of acetic acid is lower than concentrations alone indicate, and the importance of ethanol is roughly tied with two aldehydes (hexanal and 3-methylbutanal) and lower than two esters (propyl acetate and methyl acetate) (Fig. 2). Values of I<sub>2</sub> for some other esters (ethyl acetate, ethyl lactate) are not far below those for ethanol. Based on reactivity and concentration (index I<sub>3</sub>), ethanol and acetic acid have a very similar importance (Fig. 3), but otherwise relationships among compounds are similar to those based on concentration alone (Fig. 1).

With concentration, reactivity, and volatility considered (index I<sub>4</sub>), the relative importance of acetic and other acids, which are generally not highly reactive, is much lower than ethanol (Fig. 4). Conversely, two aldehydes and a single ester have values higher than ethanol.

These results can be used to quantify the relative contributions of silage VOCs to emissions and ozone formation under two hypothetical scenarios: limited emission with no depletion (Figs. 2

**Table 3**  
Volatile organic compounds previously quantified within silage, their reactivity, and volatility.

Group	Compound name	CAS number <sup>a</sup>	EBIR <sup>b</sup>	MIR <sup>c</sup>	H <sup>d</sup>
Acids	Acetic acid	64-19-7	0.20	0.66	-5.12
	Propionic acid	79-09-4	0.34	1.17	-5.00
	Butyric acid	107-92-6	0.55	1.75	-4.67
	Isobutyric acid	79-31-2	0.38	1.15	-4.92
	Isovaleric acid	503-74-2	0.96	4.11	-4.47
Alcohols	Methanol	67-56-1	0.20	0.65	-3.74
	Ethanol	64-17-5	0.57	1.45	-3.67
	1-Propanol	71-23-8	0.79	2.38	-3.54
	2-Propanol	67-63-0	0.26	0.59	-3.49
	2-Propanol <sup>e</sup>	107-18-6	2.75	11.37	-3.69
	2-Methyl-1-propanol	78-83-1	0.72	2.41	-3.36
	1-Butanol	71-36-3	0.88	2.76	-3.47
	2-Butanol	78-92-2	0.50	1.30	-3.44
	3-Methyl-1-butanol <sup>f</sup>	123-51-3	0.90	3.04	-3.22
	2-Methyl-1-butanol <sup>g</sup>	137-32-6	0.72	2.30	-3.24
	1-Pentanol <sup>g</sup>	71-41-0	0.84	2.71	-3.29
	1-Hexanol	111-27-3	0.82	2.56	-3.18
	Phenylmethanol <sup>g</sup>	100-51-6	0.82	4.98	-5.35
	2-Phenylethanol <sup>e,f</sup>	60-12-8	1.07	4.98	-5.06
	Ketones	Acetone	67-64-1	0.09	0.35
2-Butanone		78-93-3	0.37	1.43	-2.64
3-Hydroxy-2-butanone <sup>e,f</sup>		513-86-0	0.37	1.43	-3.38
Esters	Methyl acetate	79-20-9	0.04	0.07	-2.33
	Methyl butyrate <sup>g</sup>	623-42-7	0.34	1.04	-2.08
	Methyl hexanoate <sup>e,g</sup>	106-70-7	0.53	1.68	-1.83
	Methyl valerate <sup>e,g</sup>	624-24-8	0.42	1.33	-1.89
	Ethyl acetate	141-78-6	0.24	0.59	-2.20
	Ethyl butyrate	105-54-4	0.38	1.11	-1.84
	Ethyl propionate <sup>g</sup>	105-37-3	0.28	0.73	-2.06
	Ethyl lactate <sup>f</sup>	97-64-3	0.59	2.39	-2.71
	Ethyl valerate <sup>e,g</sup>	539-82-2	0.43	1.25	-1.86
	Ethyl hexanoate <sup>e,f,g</sup>	123-66-0	0.49	1.47	-1.56
	Propyl acetate	109-60-4	0.31	0.73	-2.06
	Propyl lactate <sup>e,f</sup>	616-09-1	0.35	0.99	-2.58
	Propyl butyrate <sup>g</sup>	105-66-8	0.35	0.99	-1.67
	Butyl acetate <sup>g</sup>	123-86-4	0.32	0.78	-1.94
	Butyl butyrate <sup>f,g</sup>	109-21-7	0.33	1.02	-1.56
Aldehydes	Acetaldehyde	75-07-0	1.61	6.34	-2.54
	Propionaldehyde	123-38-6	1.71	6.83	-2.51
	2-Methylpropanal <sup>f</sup>	78-84-2	1.35	5.05	-2.20
	Butyraldehyde	123-72-8	1.45	5.75	-2.33
	2-Methylbutanal <sup>e,f,g</sup>	96-17-3	1.21	4.79	-2.07
	3-Methylbutanal <sup>f</sup>	590-86-3	1.21	4.79	-1.98
	Valeraldehyde	110-62-3	1.26	4.89	-2.20
Hexanal	66-25-1	1.07	4.18	-2.07	
Heptanal	111-71-7	0.90	3.54	-1.91	

<sup>a</sup> <http://www.cas.org/content/chemical-substances/faqs>.

<sup>b</sup> Equal benefit incremental reactivity (g O<sub>3</sub> per g VOC) (Carter, 2009).

<sup>c</sup> Maximum incremental reactivity (g O<sub>3</sub> per g VOC) (Carter, 2009).

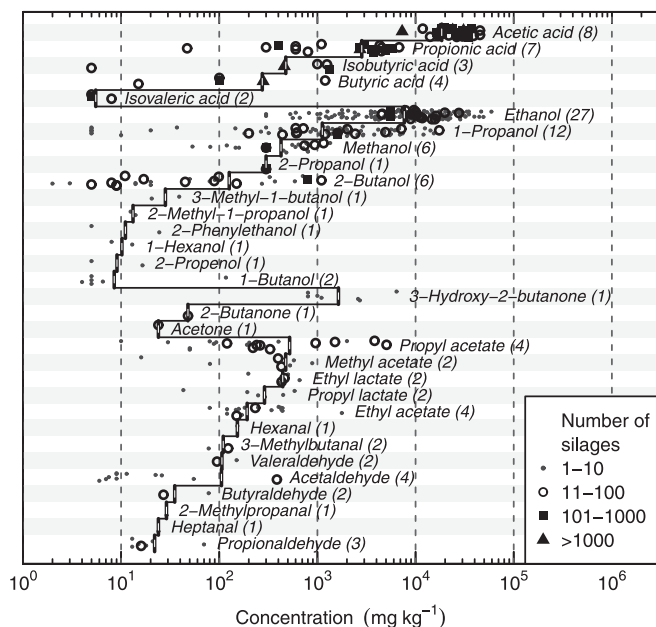
<sup>d</sup> log<sub>10</sub> of unitless Henry's law constant (gas:aqueous) (an indication of volatility).

<sup>e</sup> Reactivities estimated from values for other compounds as described in text.

<sup>f</sup> Henry's law constant is a modeled value from ChemSpider (<http://www.chemspider.com/>).

<sup>g</sup> No concentrations >10 mg kg<sup>-1</sup>, so compound was not included in analysis.

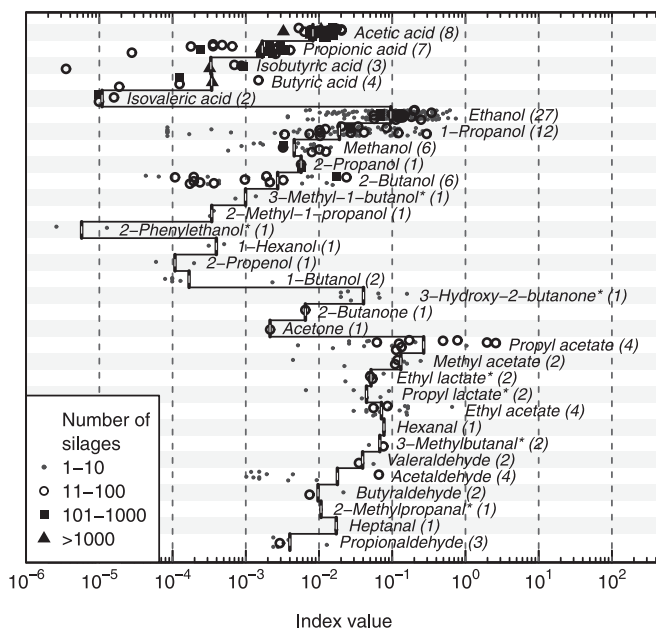
and 4) and extended exposure with complete depletion (Figs. 1 and 3), and can provide information on relative contributions under typical, intermediate scenarios. Sums of the mean and maximum concentrations of the compounds within each group are given in Table 4. Only acids and alcohols exceed 10% of the total for I<sub>3</sub>, as do alcohols, esters, and aldehydes for I<sub>4</sub>. Alcohols appear to be significant under both limited exposure with no depletion and extended exposure with complete depletion, and therefore we can conclude that they generally contribute a significant fraction of total VOC emissions and ozone formation from silage. Excluding acids, alcohols make up about 70% of the total mass of VOCs in corn silage (Table 4). Of the alcohols that have been measured, only ethanol and 1-propanol have mean values above 10% of the total for any of the indices. Most of the remaining alcohols are present at such low



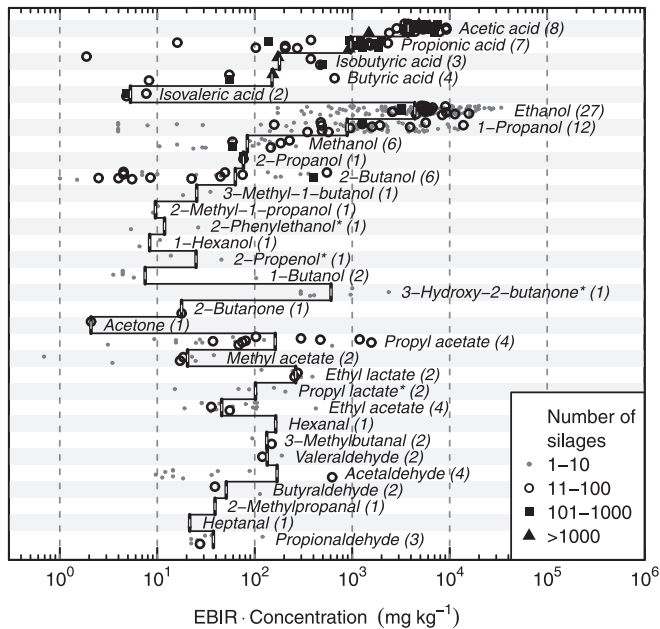
**Fig. 1.** Concentrations (index I<sub>1</sub>) of VOCs (dry matter basis) within corn silage from studies shown in Table 2. Each point represents a single observation from one study. Vertical line segments show overall means (weighted geometric means) for each compound. Numbers in parentheses are the number of studies from which data for each compound were taken. For reference, the sum of mean concentrations across all compounds was 36.0 g kg<sup>-1</sup>.

concentrations that they generally do not contribute much to VOC emission or O<sub>3</sub> production.

Assessing the contribution of other compounds to VOC emission and ozone formation is less straightforward. Ranks of emission and potential ozone formation will fall somewhere between the ranks determined from indices with and without volatility included. The

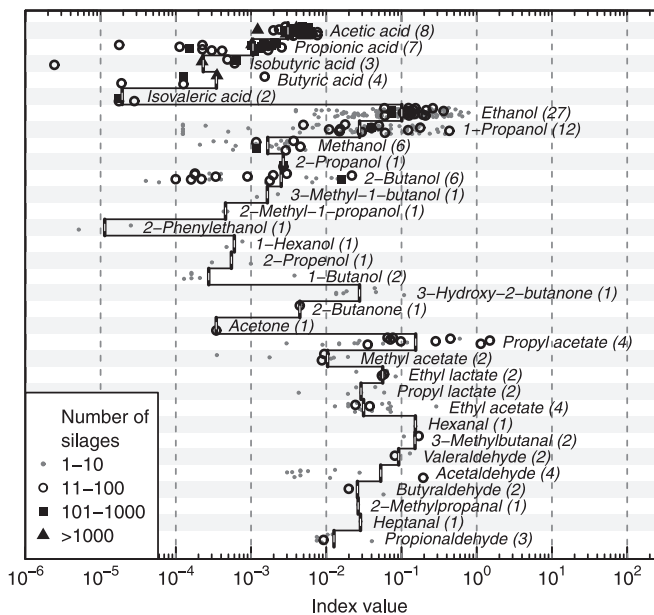


**Fig. 2.** Values of the index I<sub>2</sub> (normalized H<sub>c</sub>, product of volatility and concentration) values for VOCs within corn silage from studies shown in Table 2. Each point represents a single observation from one study. Vertical line segments show overall means (weighted geometric means) for each compound. Numbers in parentheses are the number of studies from which data for each compound were taken. Asterisks indicate compounds for which the Henry's law value is a modeled value.



**Fig. 3.** Values of the index  $I_3$  ( $rc$ , product of reactivity and concentration) values for VOCs within corn silage from studies shown in Table 2. Equal Benefit Incremental Reactivity (EBIR) was used for reactivity. Each point represents a single observation from one study. Vertical line segments show overall means (weighted geometric means) for each compound. Numbers in parentheses are the number of studies from which data for each compound were taken. Asterisks indicate compounds for which EBIR was estimated from other compounds. For reference, the sum of mean values across all compounds was  $12.6 \text{ g kg}^{-1}$ .

degree of depletion determines which indices are more accurate, and it will differ among compounds with different volatility. As discussed above (Section 3.1.2), mass balance measurements suggest that, for loose silage, 6 h alcohol emission from layers 10 cm deep is generally lower than 60% of initial concentrations. Alcohol emission from



**Fig. 4.** Values of the index  $I_4$  (normalized  $Hrc$ , product of volatility, reactivity, and concentration) values for VOCs within corn silage from studies shown in Table 2. EBIR was used for reactivity. Each point represents a single observation from one study. Vertical line segments show overall means (weighted geometric means) for each compound. Numbers in parentheses are the number of studies from which data for each compound were taken.

**Table 4**

Summed mean concentrations and importance indices for silage VOCs by group.

Group	Mean conc. ( $\text{mg kg}^{-1}$ )	Mean $I_2$	Mean $I_3$ ( $\text{mg kg}^{-1}$ )	Mean $I_4$	Max. conc. ( $\text{mg kg}^{-1}$ )	Max. $I_3$ ( $\text{mg kg}^{-1}$ )
Acids	22,058	0.01	5052	0.00	54,717	12,701
Alcohols	9732	0.13	5584	0.14	79,529	48,697
Esters	1922	0.57	593	0.28	8952	2628
Ketones	1709	0.05	625	0.03	6472	2387
Aldehydes	584	0.24	749	0.54	1014	1418
Total	36,005	1.00	12,603	1.00	150,684	67,831

Notes:  $I_2$  through  $I_4$  are indices calculated from volatility and concentration ( $I_2$ , normalized), reactivity and concentration ( $I_3$ ), and volatility, reactivity, and concentration ( $I_4$ , normalized). Maximum results are based on the maximum observed values for individual compounds.

packed silage is likely lower, due to lower porosity, as indicated by wind tunnel trials. Therefore, nearly complete depletion of alcohols is probably uncommon.

These alcohol data provide a reference point that can be used to make inferences about all compounds. Acids are less volatile than alcohols (Table 3), and so depletion by emission during normal silage management is even less likely for this group. The indices that include volatility may actually overestimate the importance of acids, because ionization and sorption are not considered. The fraction of acids in the free (un-ionized) form will decrease as depletion occurs, due to an increase in pH. Even oven drying at  $60^\circ\text{C}$  does not volatilize 100% of acetic acid or other acids present in silage (Nielsen et al., 2007). Furthermore, microbial degradation of acids may be significant over the length of time required for complete VOC volatilization. Acids, then, are unlikely to be significant contributors to ozone formation from silage emissions, except under unusual conditions that cause nearly complete depletion of all VOCs. These conditions are not impossible, and may regularly occur for some fraction of silage, e.g., wasted feed that dries out.

Conversely, relative losses of aldehydes and esters will be greater than losses of alcohols, and may approach depletion. Measurement of acetaldehyde and alcohol losses in the mass balance trials reviewed in Section 3.1.2 demonstrated this relationship; acetaldehyde loss was always greater than loss of alcohols, and exceeded 90% in some trials. Aldehydes and esters may be important when overall emission is low, and depletion is not approached, i.e., when VOC emission is minimized. It is difficult to make precise predictions on the relative contributions of VOC groups to emissions and ozone formation without more information on relative emission losses for these groups. However, the low concentrations of aldehydes and esters (compared to alcohols) constrain their potential contribution. Assuming the losses measured in the mass balance trials by Hafner et al. (2012) are representative (21%–60% loss of alcohols, 45%–92% loss of the aldehyde), alcohols typically make a much larger contribution to emissions and ozone formation, because the sum of alcohol concentrations is much higher than it is for aldehydes. The same pattern is likely to be true for esters as well. For example, with 100% emission of esters from silage with the mean concentrations given in Table 4, it would take an 11% loss of alcohols to match the ester contribution to ozone formation.

Because the records compiled in this section are based on different numbers of silages, and do not constitute a random sample of silages, they cannot be used together to quantitatively estimate variability on farms. However, data from two individual studies provide an indication of variability. Measurements of alcohols and esters for 42 corn silage samples from throughout the US (Cumberland Valley Analytical Services, 2012) generally follow a log-normal distribution, with standard deviations (of  $\log_{10}$ -transformed values) between 0.16 (methanol) and 0.75 (1-propanol). The standard deviation for ethanol is 0.34.



Tolerance intervals provide an indication of the variability expected among farms. For ethanol, 87.5% one-sided nonparametric tolerance limits are 1/2.6 and 3.1 times the median value (Wilks method using the `nptol.int` function from the tolerance package in R (Young, 2010),  $\alpha = 0.10$ ). Variability in methanol is slightly lower: the tolerance limits are 1/1.7 and 1.8 times the median. For 1-propanol, tolerance limits are much higher: 1/7.3 and 23 times the median. Intervals were difficult to estimate for esters and other alcohols because of the presence of nondetects, with the exception of ethyl lactate, which is about as variable as ethanol: the tolerance limits are 1/2.4 and 3.0 times the median.

Analysis of the acid data from Rock River Laboratory (2009) and Cumberland Valley Analytical Services (2012) show that concentrations are neither normally nor log-normally distributed. Using the method described above, tolerance limits for acetic acid are 1/1.9 and 1.5 times the median for the Rock River data set ( $n = 88,428$ ) and 1/2.8 and 2.0 times the median for the Cumberland Valley data set ( $n = 5683$ ). Assuming that these results are representative of the US as a whole, for any given VOC, 25% of corn silages may have concentrations outside a factor of 2 or more of the geometric mean, with the potential for substantially higher variability.

The variability summarized here includes not just variation among silage from different farms or silos, but also analytical error. While some studies report variability, these values almost always an estimate of variability among sampling units (e.g., farms (Raun and Kristensen, 2010) or replicate laboratory silos (Chmelova et al., 2009)), and so are larger than the random component of analytical error. This source of variability has been very small when it has been reported (standard deviations of 10% or less of mean values (Kalac and Pivnickova, 1987; Hafner et al., 2012)). It is more difficult to assess biases that may be present in individual analytical methods, which may inflate the observed variability. However, except in the case of very inaccurate methods, it seems unlikely that this source of variability is significant compared to the large variability described above.

There are some differences in the types of compounds identified in silage emissions (Table 1) and within silage (Table 3). Both approaches show that alcohols (and in particular, ethanol) are important, and that acids, aldehydes, esters, and ketones are present. Many other compounds appeared in emission measurements however, including alkanes, alkenes, and aromatic compounds. Whether these groups were not reported in the studies on VOCs within silage because the methods used were not capable of detecting them or because their concentrations are low is not clear. However, two facts strongly suggest that these other compounds do not make a major contribution to either VOC emissions or ozone formation. First, emission rates (or headspace concentrations) of compounds in these groups were generally orders of magnitude lower than alcohols (Chung et al., 2009; Howard et al., 2010; Malkina et al., 2011). (An exception to this is the significant contribution of alkenes to ozone formation from corn silage emissions reported in Howard et al. (2010).) Second, these compounds are generally highly volatile (Henry's law constants for *n*-hexane and 2-butene are about five orders of magnitude greater than the value for ethanol), which means that short-term emission measurements (such as those used in the cited studies) would overestimate their importance over the longer exposure periods that take place on farms, since they are depleted more quickly than less volatile compounds such as alcohols.

### 3.3. VOC production in silage

A key to controlling VOC emission is limiting the formation of these compounds during ensiling, but that requires knowledge of the processes responsible for producing each compound.

Unfortunately, there is little direct evidence linking a particular VOC in silage to the activity of plant enzymes, specific strains of microorganisms, or chemical reactions. Generally, changes in crop characteristics in the silo are largely attributed to microorganisms and plant enzymes based on laboratory studies with microbial strains and enzymes extracted from silages. For example, *Lactobacillus buchneri* is an obligate heterofermentative lactic acid bacterial species, and strains known to ferment lactic acid to acetic acid are used as silage additives. Various studies have found reduced lactic acid and increased acetic acid concentrations in *L. buchneri*-treated silages compared with untreated silages (Kleinschmit and Kung, 2006), but there is no direct proof that the inoculant strain produced the increased acetic acid concentrations in the treated silages. In spite of this limitation, the likely sources of the major VOCs are known.

#### 3.3.1. Acids

Acetic acid, the most concentrated VOC in silage, is produced by several groups of microorganisms during ensiling. The most prominent are lactic acid bacteria (LAB), enterobacteria, and clostridia. Lactic acid bacteria are divided into three groups: obligate homofermenters, which ferment hexose sugars to lactic acid and are incapable of fermenting pentoses; facultative heterofermenters, which ferment hexoses like homofermenters but produce phosphoketolase so they can also ferment pentoses; and obligate heterofermenters, which produce lactic acid and other products from fermenting hexoses (Pahlow et al., 2003). Most species of LAB found in silage are in the latter two categories. When these LAB ferment pentoses such as xylose or arabinose, the result is one mole of lactic acid and one mole of acetic acid per mole of pentose (McDonald et al., 1991). The obligate heterofermenters may also produce acetic acid from hexose. Depending on the LAB species and the hexose fermented, acetic acid, ethanol, and mannitol may be produced in addition to lactic acid and CO<sub>2</sub>. Some obligate heterofermenters, such as some strains of *L. buchneri*, can ferment lactic acid to acetic acid and CO<sub>2</sub> (Driehuis et al., 1999), and several *L. buchneri* strains are being sold as silage additives to increase acetic acid content of silages in order to inhibit yeast and mold growth when the silage is exposed to oxygen. When oxygen is present during active fermentation in the silo, such as due to imperfect sealing of the silo, LAB will produce less lactic acid and more acetic acid (Condon, 1987). After active fermentation is complete, many LAB strains may oxidize lactic acid to acetic acid when oxygen is present (Condon, 1987). Finally, various LAB strains can ferment organic acids such as citric and malic acids to pyruvate, and from pyruvate various products may result: lactic acid, acetic acid, ethanol, 2,3-butanediol, 3-hydroxy-2-butanone (acetoin), formic acid, and CO<sub>2</sub>, depending on the strain of LAB (McDonald et al., 1991).

Enterobacteria are frequently dominant early in the ensiling process, fermenting sugars. These bacteria produce a much broader range of final end products from pyruvate than do LAB, including acetic acid, lactic acid, succinic acid, formic acid, ethanol, 2,3-butanediol, CO<sub>2</sub>, and H<sub>2</sub>, dependent on the strain (McDonald et al., 1991). Some strains may deaminate amino acids resulting in acetic acid, butyric acid, and various minor acids such as  $\alpha$ -ketobutyric acid, phenyl propionic acid, and indolepropionic acid.

Clostridia are strict anaerobes that normally develop months after active fermentation has ended in the silo and only when silage pH has failed to reach a sufficiently low level to prevent their growth. There are three principal groups of clostridia found in silages: the *Clostridium butyricum* group that ferments a wide range of carbohydrates to butyric and acetic acids, *Clostridium tyrobutyricum* that like the *C. butyricum* group ferments lactic acid to butyric acid and some acetic acid as well as CO<sub>2</sub> and H<sub>2</sub>, and the proteolytic clostridia that ferment amino acids (Pahlow et al.,

2003). Butyric acid in silages is largely the result of the activity of the first two groups. Acetic acid is a minor product of all three clostridial groups; fermentation of some amino acids may also result in acetic acid (e.g., the deamination of aspartic acid, glutamic acid and lysine; Stickland reactions with alanine and glycine (McDonald et al., 1991)). Stickland reactions may also produce isovaleric acid from oxidation of leucine and isobutyric acid from valine. As with enterobacteria, some clostridial strains may produce other acids through amino acid deamination.

A final group that may produce acetic acid are some *Bacillus* species. *Bacillus licheniformis*, *Bacillus polymyxa* and *Bacillus coagulans* have been isolated from forages and silages and are capable of fermenting sugars (McDonald et al., 1991). Products found in either pure culture or silages include lactic acid, acetic acid, ethanol, 2,3-butanediol, and glycerol.

Propionic acid is typically more than an order of magnitude lower than acetic acid in silages. There are two known exceptions. Silages high in butyric acid from clostridial activity often have significant levels of propionic acid, due to minor fermentation pathways (McDonald et al., 1991). Occasionally, silages treated with *L. buchneri* will have significant propionic acid concentrations; the propionic acid is not an end product of *L. buchneri*, but *Lactobacillus diolivorans* can degrade a product of *L. buchneri*, 1,2-propanediol, producing propionic acid and 1-propanol (Krooneman et al., 2002). Propionic acid may also be added to silage to improve aerobic stability by inhibition of fungi (McDonald et al., 1991). Doses may reach 0.3% on a fresh mass basis, or about 9000 mg kg<sup>-1</sup>, which is about three-fold the mean value observed in the data we compiled (Fig. 1).

### 3.3.2. Alcohols

The most common alcohol in silages, ethanol, is the product of sugar fermentation by various microorganisms, but the major ones are obligate heterofermentative LAB and yeasts. One mole of glucose is fermented by heterofermentative LAB to one mole of lactic acid, one mole of ethanol, and one mole of CO<sub>2</sub> whereas fermentation by yeasts produces 2 mol of ethanol and 2 mol of CO<sub>2</sub> (McDonald et al., 1991). As discussed above, enterobacteria, dependent on strain, may produce ethanol. Clostridia have pathways permitting ethanol and butanol production (McDonald et al., 1991) although an elevated ethanol concentration is not a common feature of clostridial silages. *B. polymyxa*, when added at ensiling to grass, increased the ethanol content of the resulting silage (Woolford, 1977).

Other minor alcohols may be produced by bacteria. Some alcohols, including some branched-chain compounds, are produced by LAB through amino acid catabolism. *Lactobacillus plantarum* and other *Lactobacillus* species have been shown to produce some of the alcohols that are present at low concentrations in silage, including butanol, hexanol, 3-methyl butanol, and 2-phenylethanol (Edwards and Peterson, 1994). 1-Propanol is one of the products of *L. diolivorans* fermenting 1,2-propanediol in silages (Krooneman et al., 2002). No other identified silage species are known to produce 1-propanol. Some strains of the *C. butyricum* group produce 2-propanol and butanol (Hippe et al., 1992), but it is unclear if this occurs in silage. Similarly *C. tyrobutyricum* has been observed to produce small quantities of butanol in fermenting L-lactic acid (Hippe et al., 1992).

Methanol is not an end product associated with known silage bacterial or fungal species. However, a species not found in silage, *Clostridium thermoaceticum*, has been observed to produce methanol (Hippe et al., 1992). Because other species of this genus are found in silages, it is possible that the methanol found in silages is of bacterial origin. However, a more likely source of the methanol in silages is the activity of plant enzymes prior to ensiling. Methanol is

a product of pectin demethylation, which is most active during leaf expansion and cell wall synthesis (Fall and Benson, 1996). Methanol fluxes have been measured above standing forage crops, and those fluxes increase after mowing (Karl et al., 2001; Warneke et al., 2002; Davison et al., 2008; Graus et al., 2013). These studies have not measured methanol in the crop before and after mowing so it is unclear if the increased flux above the crop after mowing is the result of a wound response in forage crops or due to increased opportunities for methanol escape from the cut and conditioned surfaces.

### 3.3.3. Other VOCs

Much of the knowledge on biochemical pathways for production of other VOCs comes from work done on the production of fermented foods. Aldehydes are produced by LAB through sugar and amino acid catabolism. Acetaldehyde is an intermediate in the fermentation of carbohydrates by heterofermentative LAB and can be produced in large quantities by some species that lack the dehydrogenase enzyme required to reduce it to ethanol (Nollet et al., 2012). It can also be formed from threonine degradation (Konings et al., 1999). Branched-chain aldehydes, including 3-methylbutanal and 2-methylpropanal, are produced through branched-chain amino acid catabolism by LAB (van Kranenburg et al., 2002; Liu and Siezen, 2006). Additionally, researchers have speculated that an abiotic, non-enzymatic reaction, the Strecker degradation, may lead to aldehyde production during cheese production (Smit et al., 2009), and it is possible that the same reaction occurs in silage. Aldehydes can also be produced through the oxidation of alcohols, which is discussed below.

Esters may be formed through both biochemical and abiotic means. Abiotic, non-enzymatic esterification of carboxylic acids and alcohols to form esters occurs at room temperatures and would be aided by the acid conditions present in silages. Weiss (2009) measured esters in various silages and observed that ethyl lactate and ethyl acetate were strongly correlated with the ethanol contents of the silages, not the respective acids. Because ethanol concentrations are normally lower than those of lactic and acetic acids, ethanol concentration should limit the chemical reaction forming esters, providing circumstantial evidence that the esters in silage are formed chemically rather than by microorganisms. Biochemical production of esters probably occurs in silage as well. LAB have acyltransferase and esterase enzymes that produce esters from carboxylic acids and alcohols (Liu and Siezen, 2006).

3-Hydroxy-2-butanone is an expected product of some species of LAB in fermenting pyruvate. *Enterococcus* and *Pediococcus* species, in particular, are able to operate heterofermentatively when glucose levels are low, and 3-hydroxy-2-butanone is one potential product (McDonald et al., 1991). 3-Hydroxy-2-butanone is also a potential product from the fermentations of citric and malic acids by LAB. Because 3-hydroxy-2-butanone is an intermediate product between pyruvate and 2,3-butanediol, 2,3-butanediol is more likely to be observed than 3-hydroxy-2-butanone.

### 3.3.4. Changes in VOCs on exposure to oxygen

Substantial losses of acetic acid and ethanol during silo storage are unlikely in a well-sealed silo. A slow leakage of oxygen would tend to increase acetic acid concentration by LAB strains that can oxidize lactic acid to acetic as discussed earlier. However, when the silo is opened for feeding, oxygen can penetrate 1 m or more from the face (Muck and Huhnke, 1995), permitting the growth of aerobic spoilage microorganisms. The initial group of microorganisms causing heating and spoilage are generally yeasts that are tolerant of low pH and can grow on lactic acid, acetic acid, and ethanol (Pahlow et al., 2003). When yeasts initiate spoilage, invariably all three main products of silage fermentation (lactic acid, acetic acid, and ethanol)

decline rapidly and simultaneously once the yeast population is greater than  $10 \text{ cfu g}^{-1}$  silage (cfu: colony-forming units) (Courtin and Spoelstra, 1990; Muck and Pitt, 1994). How rapidly this occurs depends on the initial yeast population, silage pH and VFA concentrations, and most importantly the exposure time to oxygen before the silage is consumed. In well-managed silos, high densities and high feed out rates reduce oxygen exposure time so that yeasts rarely have the opportunity to reach such populations.

In corn silage, spoilage can be initiated by acetic acid bacteria, and a different pattern occurs (Courtin and Spoelstra, 1990). Acetic acid bacteria will oxidize ethanol, producing acetic acid until the ethanol is depleted. The acetic acid slows the growth of the acetic acid bacteria and any yeasts present, but the acetic acid bacteria will ultimately oxidize it to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . As yeasts or acetic acid bacteria consume the two principal acids, pH rises, permitting other aerobic microorganisms to grow on other soluble and insoluble components of the silage. Acetaldehyde is an intermediate in the process of ethanol oxidation, and would be expected to be produced during ethanol oxidation, although in lower quantities than acetic acid. This process occurs during the spoilage of wine by acetic acid bacteria (Bartowsky and Henschke, 2008).

One would suspect that the other VOCs would be consumed by various aerobic microorganisms too, but that has not been investigated. Acetic acid bacteria are capable of oxidizing alcohols other than ethanol, ultimately producing carboxylic acids (Gottschalk, 1986). Given sufficient time, we would expect the concentrations of all VOCs in silage exposed to air to decline due to oxidation. However, acetic acid, acetaldehyde, and other compounds that may be intermediates in oxidation pathways may temporarily increase following exposure to air.

The rate at which VOC conversion takes place in silage exposed to air may limit the effect of these conversions on VOC emission. During the six hour mass balance trials described in Section 3.1, Hafner et al. (2012) found no significant conversion of alcohols within silage during exposure to air (the maximum decline was <4%, which is comparable to the analytical error). Oxidation of ethanol has been shown to occur in corn silage over a period of days (Spoelstra et al., 1988; Woolford, 1983). However, when spoilage is more rapid, e.g., for poorly preserved silage or hot weather, it is possible that significant changes could occur over hours.

### 3.3.5. Controlling VOC production

Emission of VOCs from silage could be reduced by reducing either VOC production or relative emission (the fraction of a VOC present that is emitted). Reducing relative emission will require a better understanding of emission processes that dominate on farms, and we do not attempt to address this task here. Possible approaches for reducing production of some VOCs, based on the current understanding of VOC production in silage, are discussed below.

As described above, the majority of the VOCs are probably produced by heterofermentative LAB or undesirable microorganisms (enterobacteria, clostridia, or yeasts) directly, or, in the case of esters, indirectly. Silage additives can be used to inhibit the activity of particular microbial groups directly (through chemical inhibitors) or indirectly (through inoculants that out-compete these groups or contribute to chemical conditions that inhibit them). However, it may be difficult to reduce production of all VOCs, or even to reduce one compound or group of compounds without increasing some others. For example, reductions in acetic acid production can be affected by silage additives and by silage management. Good silo management reduces oxygen ingress, which will limit acetic acid production. But acetic acid suppresses yeast activity, so reductions in the concentration of this compound could lead to increases in ethanol production.

Homofermentative LAB inoculants will shift fermentation toward lactic acid and away from acetic acid and ethanol while reducing pH rapidly so that the activity of non-LAB species is minimized. This approach should reduce acetic acid and ethanol production by LAB, but may not reduce total ethanol production in all circumstances. If substantial sugars remain at the end of LAB fermentation, fermentation of those remaining sugars to ethanol by yeasts is possible. For example, Driehuis and van Wakselaar (2000) found that high ethanol concentrations in grass silages often occurred in crops of high sugar content. Homofermentative LAB inoculants would not address this source of ethanol. Although homofermentative LAB inoculants could reduce ethanol production in grass and legume silages, where LAB fermentation at recommended moisture contents is normally terminated by sugars being depleted, it is unlikely to consistently reduce ethanol production in corn silage.

In corn and small grain silages, fermentation by LAB is often terminated by a low pH that inhibits further growth of LAB. Under these circumstances, there is an opportunity for yeasts to consume remaining sugars and produce ethanol. Inhibition of yeasts will probably be necessary (but may not be sufficient) for reducing VOC production in corn silage. Both heterofermentative LAB inoculants and chemical additives may be useful for this purpose. Even though heterofermentative LAB produce acetic acid and some ethanol, coupled with a reduction in yeast activity, they may produce an overall negative effect on VOC production.

Published studies on the effect of bacterial inoculants on corn silage show that reductions in VOC production are possible (at least for ethanol), but also that effects are not consistent. For example, Filya and Sucu (2010) found that *L. plantarum* (a facultative heterofermentative bacterium) and *L. buchneri* (a heterofermentative bacterium), alone or in combination with other bacteria, decreased ethanol production in corn silage by 30%–40%. Not all inoculants decreased acetic acid production, however; *L. buchneri* more than doubled it. Inoculation with *L. buchneri* was also found to decrease ethanol production in corn silage about 30% (and increase acetic acid) by Tabacco et al. (2009). Reich and Kung (2010) found that *L. buchneri* in combination with *Pediococcus* spp. (homofermentative) or *L. plantarum* reduced ethanol production in corn silage by 35%–60%. All treatments increased acetic acid, at least doubling production. Conversely, Contreras-Govea et al. (2011) saw no effect of inoculation with *L. plantarum* and other LAB on ethanol concentrations in corn silage. And in a farm-level experiment, Kristensen et al. (2010) saw no effect of either *Lactobacillus pentosus* with *Pediococcus pentosaceus* (both homofermentative) or *L. buchneri* on ethanol production in corn silage. The *L. buchneri* inoculant did increase acetic acid production by more than a factor of two, however. This study was one of the few to measure 1-propanol and 2-butanol, and both of these increased by more than a factor of three relative to untreated silage. Other studies found that inoculation increases ethanol concentrations. Steidlová and Kalac (2003) measured increases (up to 3-fold) in ethanol production in response to addition of one of three inoculants: *L. plantarum*, *L. buchneri*, and a commercial inoculum with four species (*L. plantarum*, *Lactobacillus casei*, *Enterococcus faecium*, and *Pediococcus pentosaceus*). The response differed among corn varieties, and some cases increases were not observed. Tabacco et al. (2009) found a large increase in ethanol due to inoculation with *L. plantarum*. Kleinschmit et al. (2005) also found that inoculation with *L. buchneri* increased ethanol production in corn silage. And a recent meta-analysis showed that inoculation with this same species had no consistent effect on ethanol in corn silage (Kleinschmit and Kung, 2006). This review did demonstrate a clear increase in acetic acid due to inoculation with *L. buchneri*, however. For corn silage at least, bacterial inoculants do not seem to provide consistent reductions in ethanol.

Chemical additives intended to inhibit fungi or undesirable bacteria show promise for reducing ethanol production, although not all appear to have an effect. Propionic acid, for example, does not appear to reduce ethanol concentrations in silage at either 0.1% or 0.2% (all additive doses given here are on a fresh mass basis) (Kung et al., 2004; Kleinschmit et al., 2005). However, other chemicals or mixtures of chemicals have had clear effects. Addition of a commercial product consisting of 1:1 potassium sorbate and ethylenediaminetetraacetic acid (EDTA) at 0.1% reduced ethanol in corn silage by 80% compared to untreated silage (Kleinschmit et al., 2005). Teller et al. (2012) found a similar response to a 0.1% potassium sorbate treatment: 70%–90% reductions in ethanol concentrations in corn silage. Conversely, Knicky and Sporndly (2011) found that a mixture of sodium benzoate, potassium sorbate, and sodium nitrite reduced ethanol production in legume and grass silages, but not in higher dry-matter grass silage nor in corn silage.

Based on our understanding of acetic acid and ethanol production in corn silage, a combination of a homofermentative inoculant to minimize acetic acid and ethanol production by LAB and a chemical additive to suppress yeasts seems to be a promising approach to reducing VOC production, and should be evaluated. Consistently reducing silage VOC production through the use of additives may prove to be difficult. Even if a particular chemical additive does prove to be effective at consistently reducing ethanol production, its effect on VOC emission will depend on the response of other VOCs. A reduction of 80% in VOC concentrations across the board would reduce total emission by a comparable degree, but reductions in just alcohols, or even just ethanol, without reductions in other compounds would mean a much smaller reduction in the effect of silage on air quality.

Silage is an important source of feed for cattle, and a significant expense for farmers. Considerable effort has gone into developing and implementing practices that minimize losses of silage dry matter and energy from certain fermentation pathways and poor preservation. Practices for reducing VOC emission should be consistent with these practices in order to be adopted. Fortunately, practices for reducing VOC production may also reduce dry matter and energy losses. Ethanol, for example, is not considered a desirable end-product in silage and the chemical additives discussed above that may reduce it are intended to improve silage stability. Although we don't address practices that may reduce VOC emission here, those practices (such as covering silage) may also tend to contribute to high-quality silage and minimize dry matter and energy losses. In general, the importance of minimizing these losses should be kept in mind when evaluating approaches for reducing VOC emission.

#### 4. Conclusions

Based on a review of studies on silage VOC emission, concentration, and production pathways, we conclude that alcohols contribute more to corn silage total VOC emission and the resulting ozone formation than any other VOC group. Aldehydes, esters, and acids may be significant under some conditions. More research is needed in the following areas:

1. Additional measurements of VOC concentration or production in silage, including silages other than corn, and an exploration of the causes of variability (possibly climate, management, or crop characteristics).
2. Accurate on-farm measurements of VOC emission from silage and mixed feed, including an assessment of the importance of individual ensiling stages. Measurements should include cumulative emission of at least alcohols, aldehydes, esters, and acids. Such measurements could confirm the importance of

VOC groups, identify those stages that are most important for VOC emission, help assess the effects of management practices, and provide data for estimating VOC emission on a farm or larger scale.

3. Further work on understanding the sources of silage VOCs and possible approaches for reducing production is needed, including data on the effect of biological and chemical silage additives on alcohols and other significant silage VOCs.

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