Supplemental Information

Supplements "Assessing Feasibility of Bio-acidification to Reduce On-farm Ammonia Volatilization from Dairy Manure, Digestate and Urine" (SARE ONE21-402, PI: Abraham Noe-Hays)

Lab Full Trial (Long Trial): pH Behavior Over Time

The pH of manure, digestate, and urine recipes over the course of the full trial are shown in detail in Figures S1, S2, and S3. These figures illustrate the non-linear dynamics of pH changes over time, and highlight effects of temperature and culture. For example, the warm condition tended to yield a faster initial drop in pH followed by faster rebound, compared to the cold condition. In urine, culture generally resulted in a lower substrate pH, while culture had negligible effect on digestate and liquid manure. The pH of urine combined with mixtures of paper fiber and sweet whey (Figure S4) is shown separately from the other treatments, for visual clarity. We did not observe synergistic effects from sweet whey and paper fiber combinations.

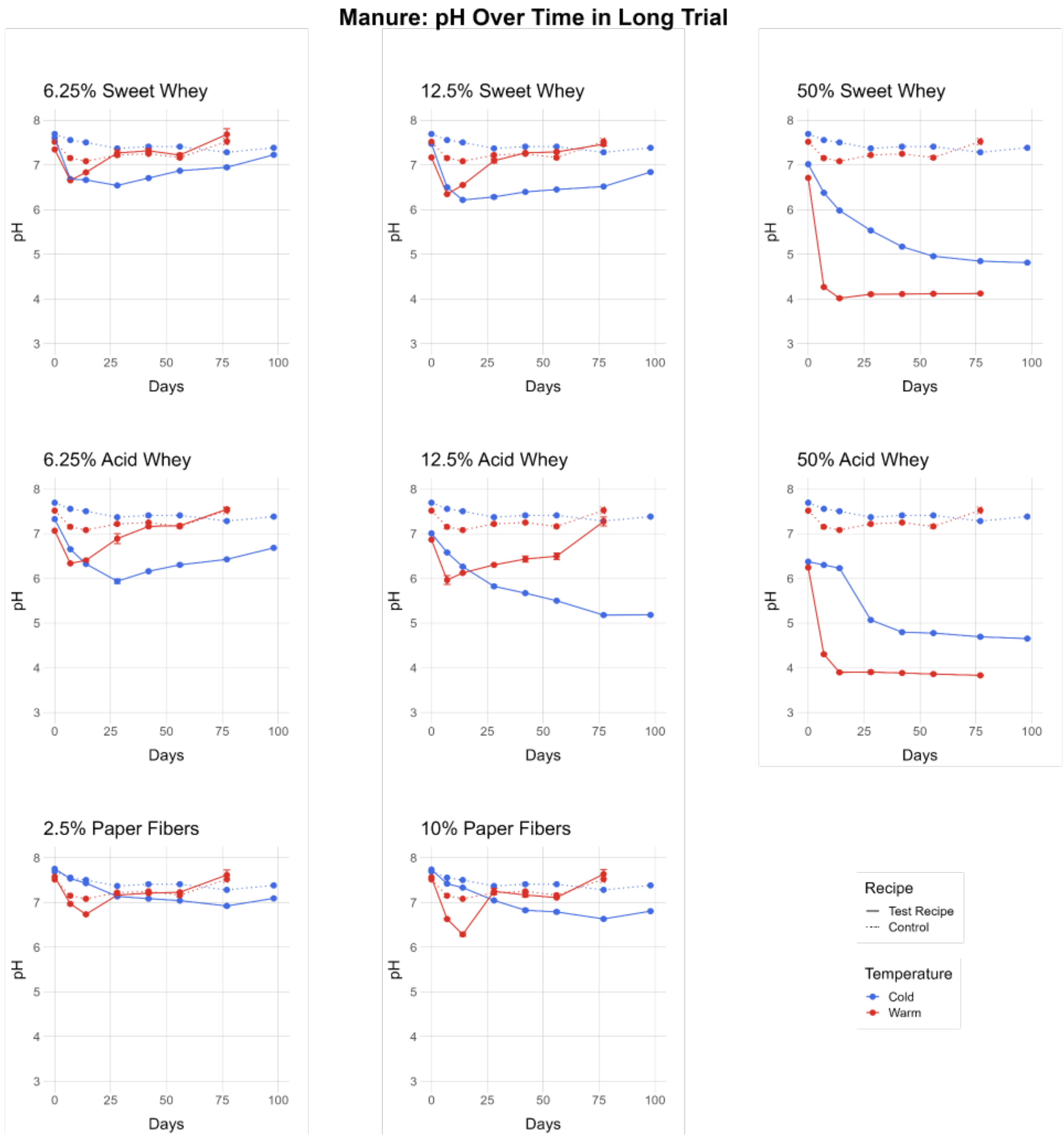

Figure S1: *The pH of manure/additive mixtures over the course of the bio-acidification experiment. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three replicate tubes, and bars represent standard error.*

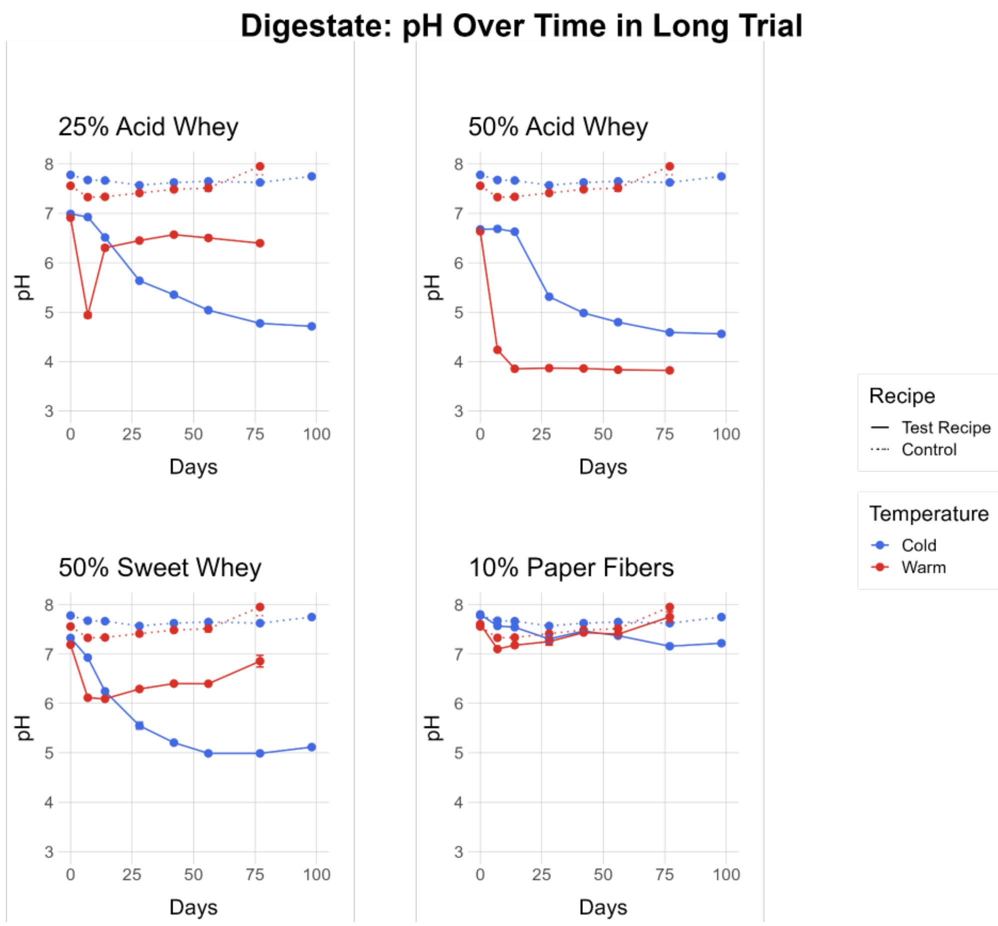

Figure S2: *The pH of digestate/additive mixtures over the course of the bio-acidification experiment. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three replicate tubes, and bars represent standard error.*

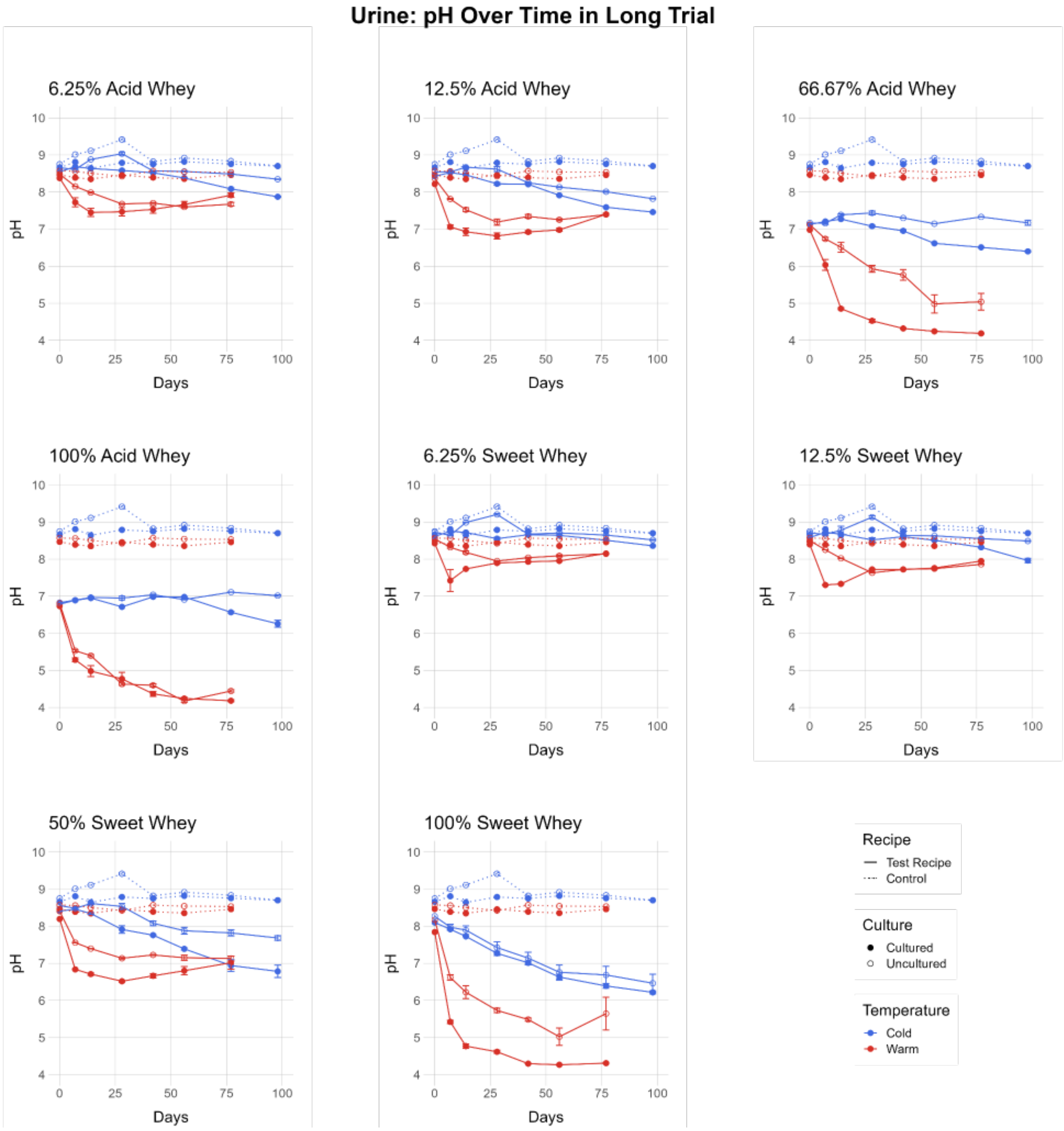

Figure S3: *The pH of urine/additive mixtures over the course of the bio-acidification experiment. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three replicate tubes, and bars represent standard error.*

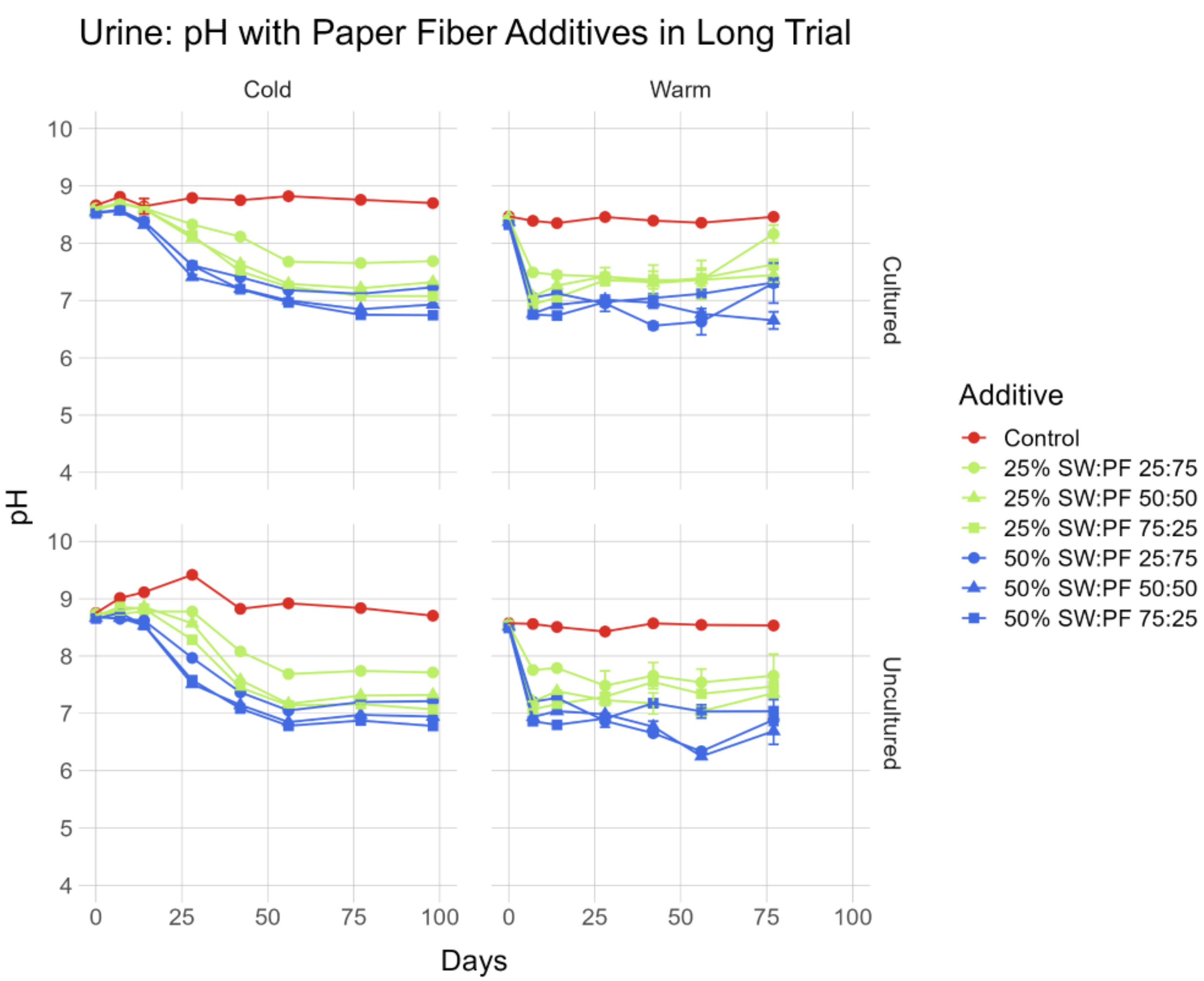

Figure S4: *The pH of urine substrates with paper fiber and sweet whey additives over the course of the bio-acidification experiment. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three trials, and bars represent standard error.*

Lab Full Trial (Long Trial): Minimum pH Reached

The lowest pH measured over the course of the full trial experiment is plotted for each recipe in Figure S5, with the pH of urine substrates with sweet whey and paper fiber-mixture additives plotted separately for visual clarity (Figure S6). From these figures, we see that a greater proportion of additive yields a lower minimum pH, though we cannot see the rate or duration of the pH drop.

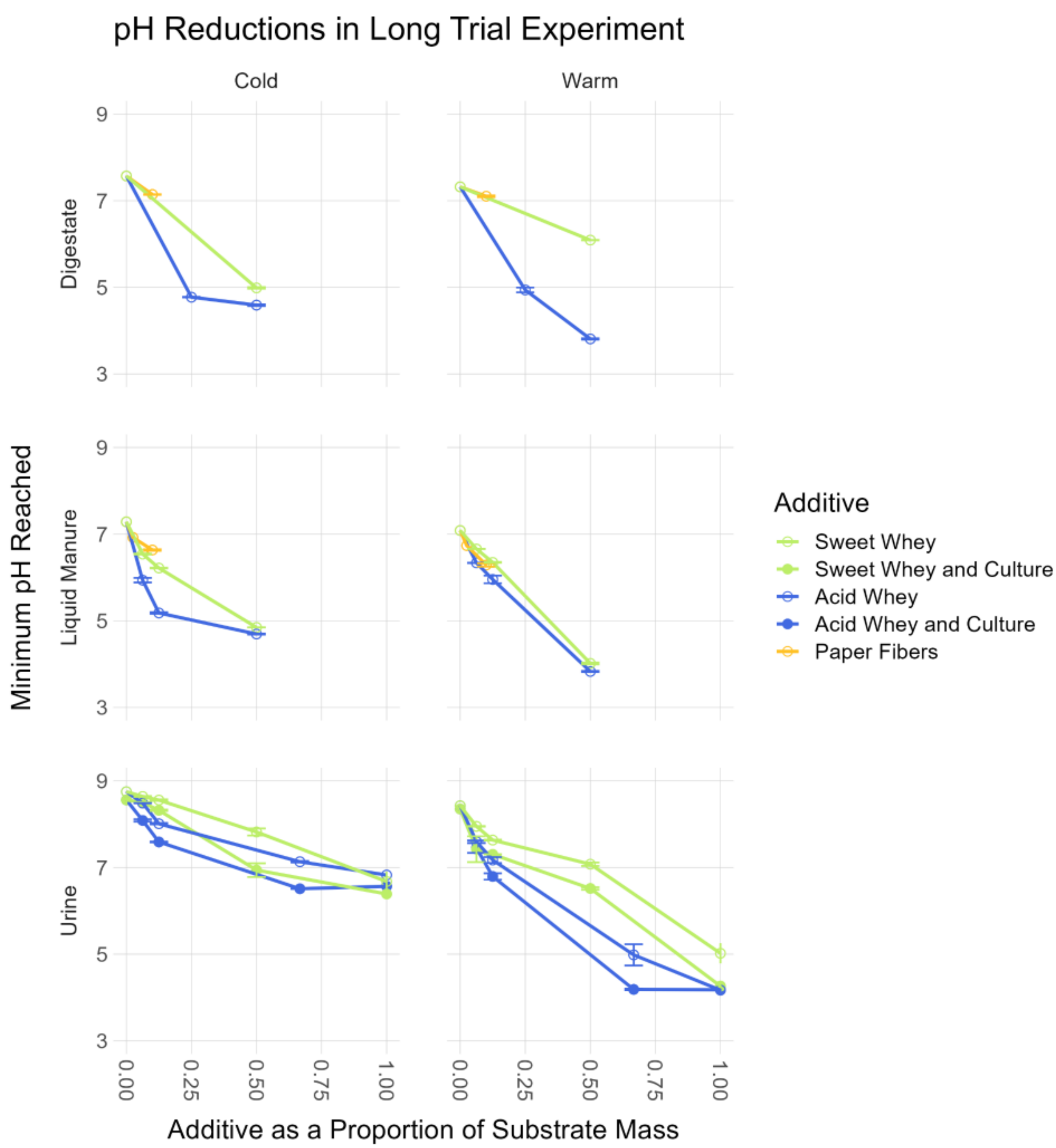

Figure S5: *The lowest pH achieved for each substrate over the long trial (liquid manure, digestate, and urine) with different ratios of additives (paper fibers, acid whey, and sweet whey, with and without culture) in long-trial experiments. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three trials, and bars represent standard error. For all substrates, ANOVA tests found pH to vary significantly by additive type (manure: $F(4,47) = 67.164$, $p < .001$; digestate: $F(3,24) = 128.68$, $p < .001$; urine: $F(5,170) = 36.264$, $p < .001$) and additive:substrate proportion (manure: $F(1,47) = 327.064$, $p < .001$; digestate: $F(1,24) = 10.73$, $p = .003$; urine: $F(1,170) = 496.82$, $p < .001$).*

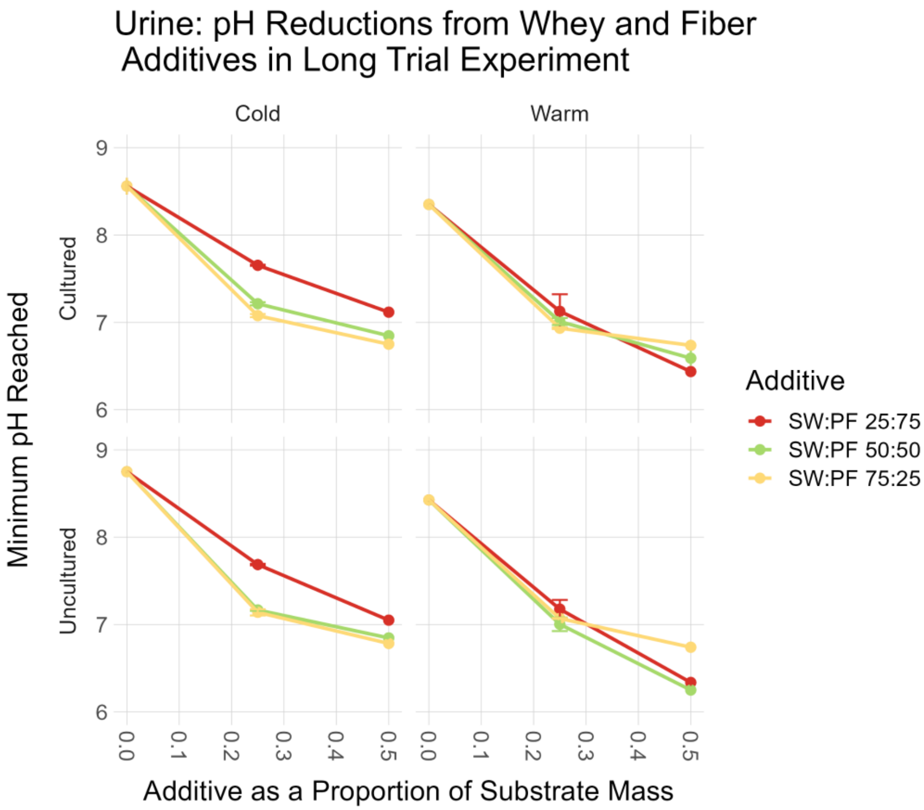

Figure S6: *The lowest pH achieved for urine substrates with different ratios of paper fiber and sweet whey additive combinations in long-trial experiments. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points each represent the average of three trials, and bars represent standard error. See Figure S5 caption for significance.*

Lab Full Trial (Long Trial): Statistical Summary

Manure recipes with the greatest pH reductions include warm 50% acid whey and sweet whey (Figure S5). Warm 50% acid whey dropped quickly to 4.30 pH by day 7, and slowly continued to drop to its lowest pH of 3.83 on day 77 (Figure S1). Warm 50% sweet whey achieved its lowest pH of 4.02 on day 14 and stayed under a pH of 4.3 for the remainder of the long trial. These large quantities of whey additives may not be practical for a farm context, however, and large pH reductions were also observed from cold 12.5% acid whey, which continued to drop in pH over the course of the long trial to a pH of 5.18, and unofficial sampling beyond the long trial found a pH of 5.06 on day 369.

In digestate, the largest pH drop was seen in warm 50% acid whey (Figure S5), which dropped to 4.24 on day 7 and continued to drop to a pH of 3.82 on day 77 (Figure S2). Yet a large pH drop is still observed at half the additive strength, as cold 25 % acid whey dropped to a pH of 6.51 on day 14 and continued to drop to a pH of 4.71 on day 98. Furthermore, unofficial testing of this sample revealed a pH of 4.43 on day 369, indicating that pH continued to drop beyond the timeline of this experiment.

In urine, the lowest pH was achieved with cultured acid whey at 100% (4.18 pH) on day 77 (Figure S5). Warm cultured acid whey also showed a large pH drop when added at smaller proportions; 12.5% additions reduced pH to 6.82 on day 24, followed by a rise in pH to 7.40 on day 77 (Figure S3). 66.67% additions showed continuous reductions in pH to 4.19 by the end of the experiment, and unofficial testing suggested that pH remained low at 4.24 on day 369. Synergistic effects were not detected from sweet whey in combination with paper fibers, and performed similarly to sweet whey additives at the same proportions (Figure S5).

Effect of additive on Minimum Substrate pH:

Our long trial analysis of substrate pH across different additives over 100 days indicated that the minimum pH achieved varied by additive (Figure S5). For all substrates, ANOVA tests found pH to vary significantly by additive type (manure: $F(4,47) = 67.164$, $p < .001$; digestate: $F(3,24) = 128.68$, $p < .001$; urine: $F(5,170) = 36.264$, $p < .001$) and additive:substrate proportion (manure: $F(1,47) = 327.064$, $p < .001$; digestate: $F(1,24) = 10.73$, $p = .003$; urine: $F(1,170) = 496.82$, $p < .001$). Acid whey appears to reduce pH more than sweet whey when added at similar proportions, and higher proportions of additive to substrate corresponded to lower pH values.

Ammonia Volatilization Jar Tests: Statistical Summary

Liquid Manure: Effects of Additive on Volatilization Rates

For manure substrates, 12.5% acid whey additions were found to stop all volatilization for 4 days (Figure 7) before a rapid release of ammonia beginning after day 6. Additions of 6.25% acid whey and 10% paper fibers also showed strong reductions in ammonia loss at day 4, losing respectively 70% and 57% less ammonia than the control. ANOVA tests found volatilization rates varied significantly by additive for all days (8 hours: $F(6,14) = 80.42$, $p < .001$; day 1: $F(6,14) = 132.5$, $p < .001$; day 2: $F(6,14) = 495.3$, $p < .001$; day 4: $F(6,14) = 433.1$, $p < .001$; day 6: $F(6,14) = 262.7$, $p < .001$; day 12: $F(6,14) = 23.93$, $p < .001$; day 20: $F(6,14) = 8.372$, $p < .001$).

A Tukey's HSD test for multiple comparisons (confidence level = 0.95) showed significant pairwise differences in ammonia volatilization for all manure additives for all days ($p < 0.05$) with many exceptions: Volatilization from controls did not differ from 2.5% paper fibers after day 6, 12.5% sweet whey after day 12, nor 12.5% acid whey and 6.25% sweet whey on day 20. Though 12.5% acid whey additions had significantly lower ammonia volatilization than all recipes for the first 6 days, it showed a rapid release of ammonia such that retention was not improved relative to 6.25% acid whey and 10% paper fibers after day 12, and by day 20 it did not vary from 6.25% or 12.5% sweet whey, or 2.5% paper fibers. Volatilization from 6.25% sweet whey did not differ from 2.5% paper fibers for all days except day 6, from 12.5% sweet whey after day 4, and from 6.25% acid whey and 10% paper fibers on day 20. Additionally, volatilization from 10% paper fibers did not vary from 6.25% acid whey on days 1, 2, 12, and 20, and volatilization from 2.5% paper fibers did not vary from 12.5% sweet whey after day 6.

Digestate: Effects of Additive on Volatilization Rates

In digestate substrates, 50% sweet whey and 25% acid whey respectively lost 61% and 75% less ammonia than the non-acidified control (Figure 8). ANOVA tests found volatilization rates to vary significantly between additives for all days (8 hours: $F(2,6) = 281.2$, $p < .001$; day 1: $F(2,6) = 253.5$, $p < .001$; day 2: $F(2,6) = 321.5$, $p < .001$; Day 4: $F(2,6) = 192.3$, $p < .001$; day 6: $F(2,6) = 135.4$, $p < .001$; day 12: $F(2,6) = 49.86$, $p < .001$; day 20: $F(2,6) = 27.27$, $p < .001$), and a Tukey's HSD test for multiple comparisons (confidence level = .95) showed significant pairwise differences between all recipes for all sample days ($p < .05$).

Urine: Effects of Additive on Volatilization Rates

For cultured urine substrates, 100% acid whey performed best, with only 1% of the volatilization seen in the control samples by day 20 (Figure 9). Yet a 1:1 ratio of acid whey and urine may not be practical given limited storage space and access to whey, and these trials showed that 12.5% acid whey, 6.25% acid whey, and 6.25% sweet whey still reduced ammonia volatilization by 57%, 29%, and 15%, respectively, compared to control samples at the 4-days mark.

ANOVA tests indicated that ammonia volatilization in urine varies significantly by additive for all days (8 hours: $F(4,10) = 96.91$, $p < .001$; day 1: $F(4,10) = 166.2$, $p < .001$; day 2: $F(4,10) = 465.9$, $p < .001$; Day 4: $F(4,9) = 476.4$, $p < .001$; day 6: $F(4,9) = 335.2$, $p < .001$; day 12: $F(4,9) = 157.3$, $p <$

.001; day 20: $F(4,9) = 192.6$, $p < .001$). Tukey's HSD test for multiple comparisons (confidence level = 0.95) showed significant pairwise differences between all recipes for all days ($p < 0.05$) except that 6.25% sweet whey was not significantly different from the urine control for days 12 and 20, and did not vary significantly from 6.25% acid whey on days 1, 12 and 20. A sample of 6.25% sweet whey was lost on day 4, which may account for a lack of statistical power in comparisons made after that time.

Effect of Starting pH on Ammonia Volatilization

For all substrates tested in volatilization trials, lower initial pH values appear to correlate with lower ammonia volatilization, though we did not test this relationship for statistical significance. In manure, strong reductions in volatilization through day 4 from additions of 6.25% and 12.5% acid whey and 10% paper fibers correspond with low initial pH values of 6.59, 5.21, and 6.92, respectively (Figure S7). An anomaly is seen with additions of 12.5% sweet whey, which retained less nitrogen than 10% paper fibers on day 4, despite a lower starting pH of 6.83. Similar positive trends between pH and ammonia loss are observed in digestate and urine substrates (Figure S8, Figure S9). These findings agree with established theory that ammonia volatilization is reduced at a lower pH (Freney et al. 1983).

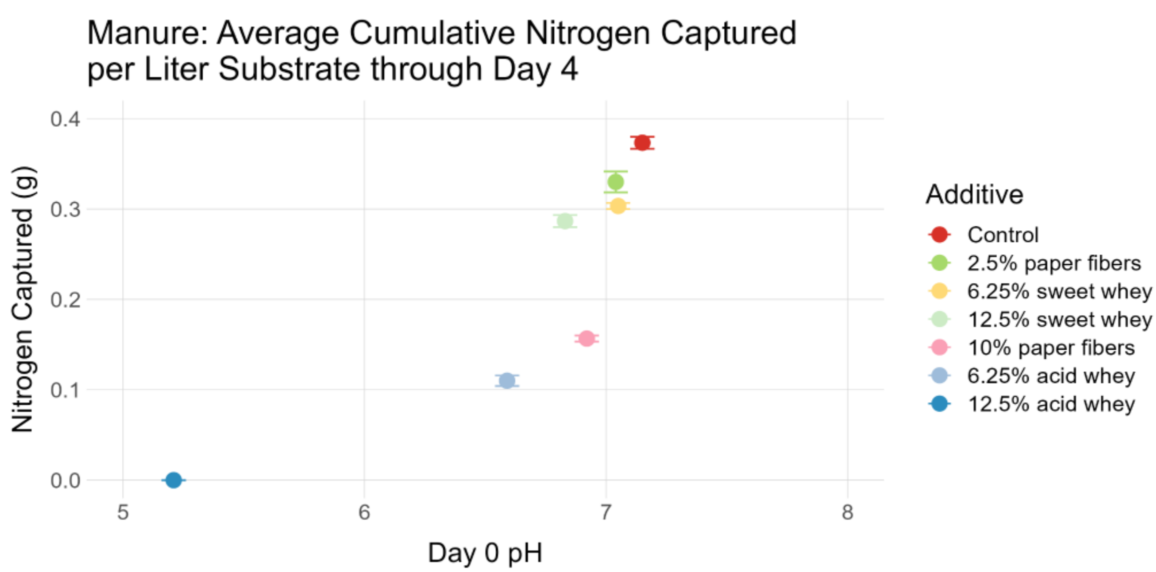

Figure S7: *This scatterplot shows the cumulative ammonia volatilized through day 4 from bio-acidified liquid manure samples in relation to their pH measured on day 0 of the experiment. Additive percentages represent the amount of additive as a proportion of manure mass (50% indicates a manure:additive ratio of 2:1). Points represent the average of three replicates, and bars represent standard error.*

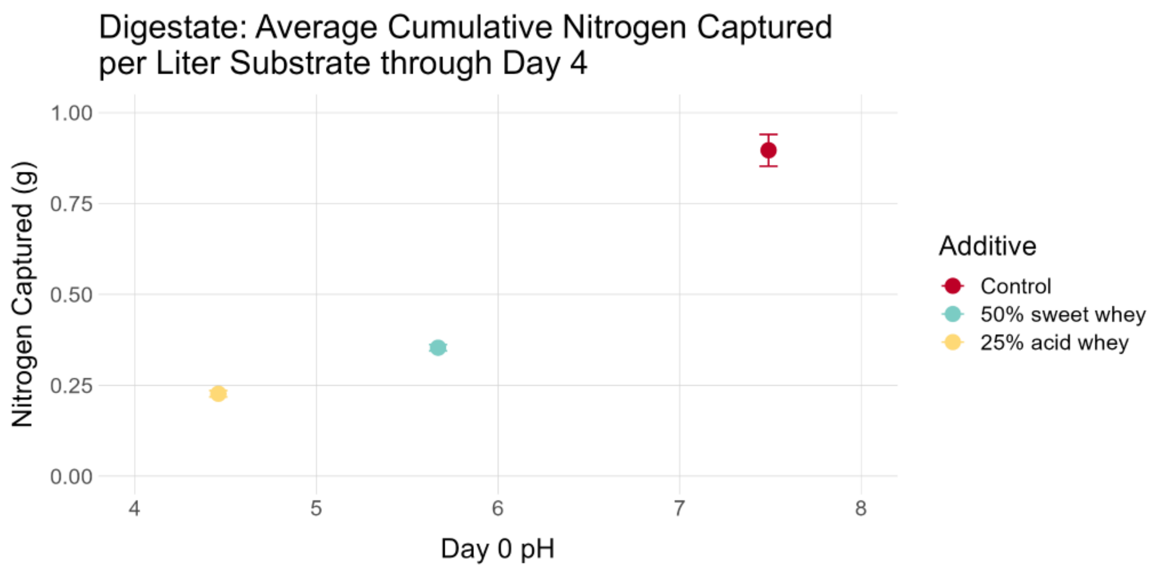

Figure S8: *A scatterplot shows the cumulative ammonia volatilized through day 4 from bio-acidified digestate samples in relation to their pH measured on day 0 of the experiment. Additive percentages represent the amount of additive as a proportion of digestate mass (50% indicates a digestate:additive ratio of 2:1). Points represent the average of three trials, and bars represent standard error.*

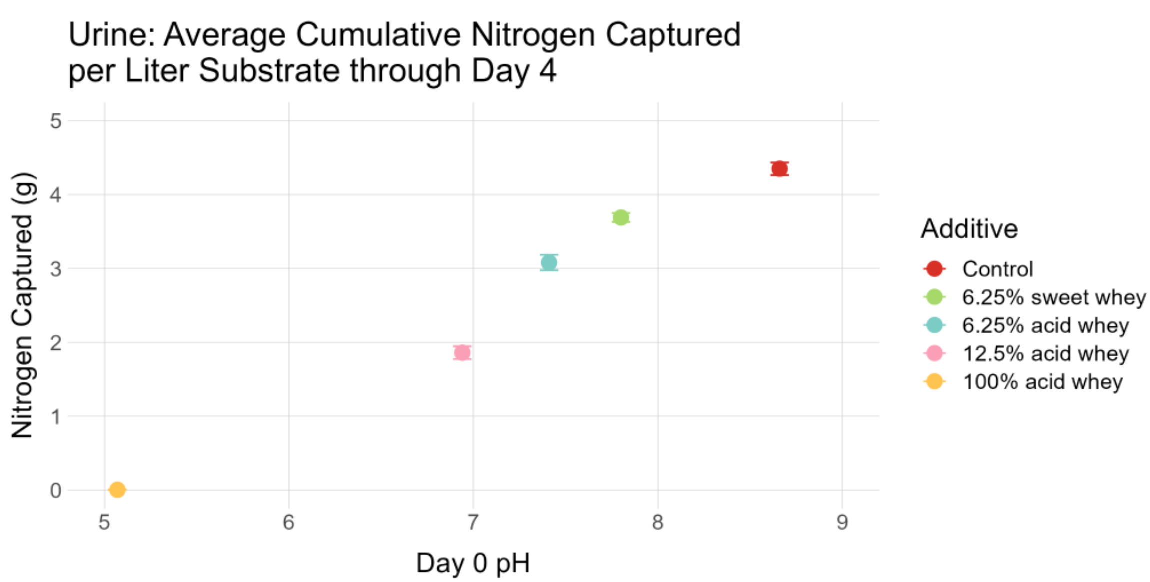

Figure S9: *A scatterplot shows the cumulative ammonia volatilized through day 4 from bio-acidified urine samples in relation to their pH measured on day 0 of the experiment. Additive percentages represent the amount of additive as a proportion of urine mass (50% indicates an urine:additive ratio of 2:1). Points represent the average of three trials, and bars represent standard error.*

Sources Cited

Freney, J. R., Simpson, J. R., & Denmead, O. T. (1983). Volatilization of ammonia. In J. R. Freney & J. R. Simpson (Eds.), Gaseous Loss of Nitrogen from Plant-Soil Systems (pp. 1–32). Springer Netherlands. https://doi.org/10.1007/978-94-017-1662-8_1