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Heather Darby
University of Vermont

Susan Monahan
University of Vermont

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Dr. Heather Darby, UVM Extension Agronomist
Susan Monahan, UVM Extension Crops and Soils Technician

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ENHANCING FORAGES WITH NUTRIENT DENSE SPRAYS-FINAL REPORT

Dr. Heather Darby, University of Vermont Extension
Heather.Darby[at]uvm.edu

The purpose of the Nutrient Dense Spray (NDS) trial was to evaluate the impact of nutrient dense foliar sprays on perennial forage yield, quality, and fatty acid concentrations. The nutrient spray program was developed by Advancing Eco-Agriculture (Middlefield, OH) and consisted of five foliar sprays specifically recommended for the farms participating in this study. The recommended foliar applications were evaluated over 3 growing seasons. In the third year of the study, a sixth foliar spray, 'Sea Shield' was added to the treatments. The recommended spray program included applications of Rejuvenate in the early spring and late fall, and a combination of PhotoMag, Phosphorus, Potassium, MicroPak, and Sea Shield applied in the spring and after each harvest of hay or graze (Table 1). This study was conducted based on farmer interest in enhancing nutrient density of forages through foliar sprays and was funded by the Lattner Family Foundation. Any reference to commercial products, trade names or brand names is for information only, and no endorsement or approval is intended.

Table 1. Information on Advancing Eco-Agriculture nutrient dense sprays.[1]

Spray	What is it?	What does it do?
Rejuvenate	humic substance, carbohydrates, sea minerals	stimulates soil microbial life
PhotoMag	magnesium, sulfur, boron, cobalt, sea minerals	promotes chlorophyll and sugar production
Phosphorus	mined phosphate ore	improves photosynthesis and plant root vigor
Potassium	mined potassium sulfate	improves storability
MicroPak	boron, zinc, manganese, copper, cobalt, molybdenum, sulfur	enhances sugar translocation, root strength, and plant immunity
Sea Shield[2]	crab and shrimp shell concentrate	enhance plant health and immune response

[1]Information gathered from the Advancing Eco-Agriculture website: advancingecoag.com.
[2]Sea Shield was only applied in the 3rd year of the project (2014).

MATERIALS AND METHODS

Forages were sprayed with nutrient dense sprays at two locations: Shelburne Farms in Shelburne, VT and Butterworks Farm in Westfield, VT. Both hayfields had been in native grass/legume mixture for numerous years. The nutrient recommendations from Advancing Eco-Agriculture are listed in Table 2. In order to understand what may cause a response, if any, we compared the recommended spray regime ('All') to individual components, as well as a control of water. The experimental design was a randomized complete block with four replications.

Table 2. Timing and amount of fertility and Nutrient Dense Spray applications recommended for forages in this study.

Timing	Recommendations (per acre)
Early spring	3 tons compost, 20 lb. Borate, and 5 lbs. Zinc sulfate, 2 gal. Rejuvenate, 1 gal. Sea Shield [1]
After each harvest	1 gal. PhotoMag, 1 gal. Phosphorus, 1 quart Potassium, 2 quarts MicroPak, 2 quarts Sea Shield [1]
Fall, post-harvest	6 quarts Rejuvenate, 2-3 tons compost

[1]Sea Shield was only applied in the 3rd year of the project (2014).

Six by ten foot plots were established in existing hay fields in 2012. The same plots were used in 2013 and 2014. In 2014, large strip plots were also included in the study to rule out the possibility of drift or leaching from neighboring plots. The large strip plots were 11' x 55'. All plots were harvested with a BCS sickle bar mower (Portland, OR), raked by hand, gathered and weighed on a platform scale. Harvest and spray dates are listed in Table 3. A subsample was dried at 40^{o} C and weighed to determine dry matter. Oven dry samples were coarsely ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ), finely ground with a UDY cyclone mill with a 1 mm screen (Seedburo, Des Plaines, IL) and analyzed with an NIRS (Near Infrared Reflectance Spectroscopy) DS2500 Feed and Forage analyzer (Foss, Eden Prairie, MN) at the University of Vermont Cereal Testing Lab (Burlington, VT). Results were analyzed with an analysis of variance in SAS (Cary, NC).

Table 3. Harvest and spray dates at each location.

Treatment	Butterworks Farm			Shelburne Farms		
	2012	2013	2014*	2012	2013	2014
Spray Rejuvenate	18-Apr	1-May	8-May	19-Apr	30-Apr	7 & 9-May
Spray All Treatments	16-May	1-May	8-May	24-Apr (B, Zn)	30-Apr	27-May
1st Cut	31-May	4-Jun	6-Jun	17-May	22-May	27-May
Spray All Treatments	12-Jun	12-Jun	17-Jun	29-May	30-May	4-Jun
2nd Cut	9-Jul	3-Jul	3-Jul	21-Jun	18-Jun	30-Jun
Spray All Treatments	18-Jul	16-Jul	14-Jul	5-Jul	2-Jul	8-Jul
3rd Cut	21-Aug	9-Aug	6-Aug	27-Jul	6-Aug	6-Aug
Spray All Treatments	28-Aug	20-Aug		7-Aug	19-Aug	
Spray Rejuvenate	9-Oct	3-Oct		9-Oct	1-Oct	

*Butterworks Farm harvested a 4th cut in 2014 on 24-Sep.

Forage samples were dried, ground and analyzed for quality characteristics including crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and various other nutrients. The Nonstructural Carbohydrates (NSC) were calculated from forage analysis data. Mixtures of true proteins, composed of amino acids and non-protein nitrogen make up the crude protein (CP) content of forages. The bulky characteristics of forage come from fiber. Forage feeding values are negatively associated with fiber since the less digestible portions of the plant are contained in the fiber fraction. The detergent fiber analysis system separates forages into two parts: cell contents, which include sugars, starches, proteins, non-protein nitrogen, fats and other highly digestible compounds; and the less digestible components found in the fiber fraction. The total fiber content of forage is contained in the neutral detergent fiber (NDF). Chemically, this fraction includes cellulose, hemicellulose and lignin. Recently, forage testing laboratories have begun to evaluate forages for NDF digestibility. Evaluation of forages and other feedstuffs for NDF digestibility is being conducted to aid prediction of feed energy content and animal performance. Research has demonstrated that lactating dairy cows will eat more dry matter and produce more milk when fed forages with optimum NDF digestibility. Forages with increased NDF digestibility (NDFD) will result in higher energy values, and perhaps more importantly, increased forage intakes. Forage NDF digestibility can range from 20 – 80%. The NSC or non-fiber carbohydrates (NFC) include starch, sugars and pectins.

Mineral analysis was determined by wet chemistry at Cumberland Valley Analytical Services (Hagerstown, MD). Metals and Other Elements in Plants (985.01). Official Methods of Analysis, 17th edition. 2000. Association of Official Analytical Chemists. Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484. Modifications include: Ash 0.35g sample for 1 hr at 535°C. Digest in open crucibles for 20 min in 15% nitric acid on hotplate. Samples diluted to 50ml and analyzed on ICP.

Fatty acid content and profile of the feed samples were analyzed using a modified version of the direct transesterification method developed by Sukhija and Palmquist (1988). In brief, 1 mL of internal standard (1 mg C13:0

TAG/mL acetone), 2 mL of toluene, and 2 mL of 2% methanolic H_2SO_4 acid were added to 500 mg of ground feed composites samples. The solution was heated at 50°C overnight. After cooling the samples to room temperature, 5 mL of 6% $KHCO_3$ solution and 1 mL of hexane were added. The samples were mixed and centrifuged at 500 x *g* for 5 min. The resulting hexane layer was dried and cleaned over a mixture of Na_2SO_4 and charcoal. An aliquot of the solution, containing the fatty acid methyl esters (FAME), was taken for GLC analysis. The analysis of FAME extracts was performed on a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a split injector, a flame ionization detector, an autosampler (model AOC-20s; Shimadzu), and a 100 m CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Varian Inc., Palo Alto, CA) The injector and detector were both maintained at 250°C. Hydrogen was used as carrier gas at a linear velocity of 30 cm/sec. The sample injection volume was 1 μL at a split ratio of 1:50. The oven program used was: initial temperature of 45°C held for 4 min, programmed at 13°C/min to 175°C held for 27 min, then programmed at 4°C/min to 215°C held for 35 min. Integration and quantification was based on the FID response and achieved with GC solution software (version 2.30.00, Shimadzu, Kyoto, Japan). Identification of FAME was accomplished by comparison of relative retention times with commercial FAME standards. Total fatty acid content was determined using C13:0 as an internal standard. The fatty acid results were expressed as percentages (weight/weight) of fatty acids detected with a chain length between 10 and 24 carbon atoms. The lowest level of detection was <0.001g/100g fatty acids and is reported as not detectable (ND).

Variations in yield and quality can occur because of variations in genetics, soil, weather and other growing conditions. Statistical analysis makes it possible to determine whether a difference among varieties is real, or whether it might have occurred due to other variations in the field. At the bottom of each table, a LSD value is presented for each variable (i.e. yield). Least Significant differences (LSD's) at the 10% level of probability are shown, unless otherwise indicated. Where the difference between two treatments within a column is equal to or greater than the LSD value at the bottom of the column, you can be sure in 9 out of 10 chances that there is a real difference between the two varieties. Treatments that were not significantly lower in performance than the highest value in a particular column are indicated with an asterisk. In the following example, A is significantly different from C but not from B. The difference between A and B is equal to 1.5 which is less than the LSD value of 2.0. This means that these varieties did not differ in yield. The difference between A and C is equal to 3.0 which is greater than the LSD value of 2.0. This means that the yields of these varieties were significantly different from one another. The asterisk indicates that B was not significantly lower than the top yielding variety.

Variety	**Yield**
A	6.0
B	7.5*
C	9.0*
LSD	**2.0**

RESULTS AND DISCUSSION

Seasonal precipitation and temperature recorded at weather stations in close proximity to Westfield and Shelburne, VT from 2012 to 2014 are reported in Tables 4-6. In 2012, the temperature and precipitation in Westfield was close to the 30-year average. There were a total of 5530 GDD (growing degree days), 134 GDD above average. May, August and October were warmer than average in Westfield, with less rain in July and August. In Shelburne, monthly temperatures were above the 30-year average every month of the growing season. There were a total of 6488 GDD, 639 GDD above average. Warmer temperatures in Shelburne resulted in earlier harvests of 2nd and 3rd cut hay.

Table 4. Seasonal weather data collected near Westfield and Shelburne, VT, 2012.

Westfield*	Apr	May	Jun	Jul	Aug	Sep	Oct
Average Temperature (F)	41.8	56.7	63.0	67.9	68.1	56.9	48.8
Departure from Normal	-0.9	1.9	-0.8	-0.1	2.0	-0.6	4.0
Precipitation (inches)	3.2	3.6	4.0	3.6	2.8	6.4	4.2
Departure from Normal	0.4	0.0	0.0	-0.7	-1.8	2.9	0.2
Growing Degree Days (base 32)	336	769	928	1112	1119	747	519
Departure from Normal	4	64	-25	-4	63	-41	73

Shelburne*	Apr	May	Jun	Jul	Aug	Sep	Oct
Average Temperature (F)	46.1	61.6	67.8	73.0	72.0	61.9	52.9
Departure from Normal	1.3	5.2	2.0	2.4	3.2	1.4	4.8
Precipitation (inches)	2.8	4.4	3.2	3.8	2.9	5.36	5.04
Departure from Normal	0.0	0.9	-0.5	-0.4	-1.0	1.72	1.44
Growing Degree Days (base 32)	435	917	1072	1271	1241	925	627
Departure from Normal	51	161	58	73	102	68	126

*Data compiled from Northeast Regional Climate Center data from weather stations in Newport, VT and Burlington, VT. Historical averages for 30 years of NOAA data (1981-2010).

In 2013, the temperature and precipitation in Westfield was also close to the 30-year average. There were a total of 5243 GDDs, 112 GDDs below average. May, July, and October were warmer than average in Westfield, with substantially more rain in May, June, July and September. In Shelburne, monthly temperatures were above the 30-year average every month of the growing season except September. There were a total of 6176 GDDs, 323 GDDs above average. Warmer temperatures in Shelburne resulted in early forage harvests. In May and June, it rained about 6 inches more than normal in Westfield and 11.5 inches more than normal in Shelburne.

Table 5. Seasonal weather data collected near Westfield and Shelburne, VT, 2013.

Westfield*	Apr	May	Jun	Jul	Aug	Sep	Oct
Average Temperature (F)	39.4	55.7	62.2	69.3	64.6	56.5	47.4
Departure from Normal	-3.2	0.9	-1.6	1.3	-1.5	-1.8	1.0
Precipitation (inches)	2.78	6.53	7.08	7.29	2.78	6.79	2.46
Departure from Normal	-0.03	2.86	3.12	2.96	-1.83	3.41	-1.64
Growing Degree Days (base 32)	221	736	906	1156	1012	735	477
Departure from Normal	-102	26	-48	84	-45	-56	29

Shelburne*	Apr	May	Jun	Jul	Aug	Sep	Oct
Average Temperature (F)	44.8	60.7	66.5	73.8	69.4	60.2	51.7
Departure from Normal	0.0	4.3	0.7	3.2	0.6	-0.4	3.5
Precipitation (inches)	2.05	8.74	9.86	4.49	3.07	4.74	2.59
Departure from Normal	-0.77	5.29	6.17	0.34	-0.84	1.10	-1.01
Growing Degree Days (base 32)	383	890	1034	1253	1161	846	609
Departure from Normal	-1	133	20	54	22	-12	107

*Data compiled from Northeast Regional Climate Center data from weather stations in Newport, VT and Burlington, VT. Historical averages for 30 years of NOAA data (1981-2010).

In 2014, the temperature in Westfield was below the 30-year average for the growing season, while precipitation was above average. There were a total of 4694 GDDs, which is 222 GDDs below the average. In Shelburne, monthly temperatures were above the 30-year average for every month of the growing season except April. There were a total of 5567 GDDs, 226 GDDs above average. Warmer temperatures in Shelburne contributed to the earlier harvests of hay. There was over 3 inches of precipitation above the 30-year normal for April through July. However, August and September were dry, almost 4 inches below than the 30-year normal.

Table 6. Seasonal weather data collected near Westfield and Shelburne, VT, 2014.

Westfield*	Apr	May	Jun	Jul	Aug	Sep
Average Temperature (F)	39.4	53.6	62.9	67.2	64.6	57.4
Departure from Normal	-3.2	-1.2	-0.9	-0.8	-1.5	-0.9
Precipitation (inches)	3.04	5.39	4.45	5.85	4.83	2.73
Departure from Normal	0.23	1.72	0.49	1.52	0.22	-0.65
Growing Degree Days (base 32)	222	670	927	1091	1012	762
Departure from Normal	-101	-40	-27	19	-45	-28

Shelburne*	Apr	May	Jun	Jul	Aug	Sep
Average Temperature (F)	44.6	58.9	68.2	71.5	69.0	62.0
Departure from Normal	-0.20	2.60	2.40	0.90	0.20	1.50
Precipitation (inches)	3.66	3.94	4.35	5.54	2.05	1.63
Departure from Normal	0.84	0.49	0.66	1.38	-1.86	-2.01
Growing Degree Days (base 32)	378	834	1085	1223	1145	902
Departure from Normal	-5	81	71	26	6	45

*Data compiled from Northeast Regional Climate Center data from weather stations in Newport, VT and Burlington, VT. Historical averages for 30 years of NOAA data (1981-2010).

Impact of Nutrient Dense Spray on Forage Yield and Quality

Overall, there were no significant differences in yield or quality based on the different treatments of the NDS (Table 7). This is in agreement with what we saw each year of the trial. The NDS did not increase yield, protein, or other basic forage quality parameters. It is unclear as to why the NDS treatments did not increase perennial forage performance. It could be related to initial soil fertility status of the collaborating farms. When we evaluated the data by cut, however, there were significant differences (Table 8). In general first cut of forage yielded the highest and third cut had the highest quality (crude protein, lowest fiber content, high non-fiber carbohydrates, and the highest digestible NDF). The trends observed by cut were similar to what most farms observe in perennial forages.

Table 7. Yield and quality of forages treated with Nutrient Dense Sprays, averaged across 3 years and 2 locations.

	Yield	CP	ADF	NDF	Starch	NFC	NDFD
	lbs ac^{-1}	%	%	%	%	%	% of NDF
All	1865	17.4	29.8	53.8	2.0	24.7	48.9
Control	1965	17.5	29.5	53.2	2.1	25.2	48.9
MicroPak	1914	17.3	29.7	53.8	2.0	24.9	48.6
Phosphorus	1888	17.3	29.5	53.6	2.0	25.2	49.0
PhotoMag	1975	17.6	29.6	53.3	2.0	25.2	49.0
Potassium	1882	17.4	29.8	53.8	1.9	25.0	48.9
Rejuvenate	1968	17.4	29.7	54.1	1.9	24.6	49.1
Trial Mean	1849	21.017.5	17.529.5 29.529.5	52.4 52.448.47	48.72.12.12.1	25.9 25.925.9	48.7
Tukey-Kramer (p< 0.10)	NS	NS	NS	NS	NS	NS	NS

NS – Not Significant, none of the variables were significantly different from one another.

Table 8. Yield and quality of forages treated with Nutrient Dense Spray by cut.

	Yield	CP	ADF	NDF	Starch	NFC	NDFD	
	lbs ac^{-1}	%	%	%	%	%	% of NDF	
1st cut	**2689***	15.9	30.6	58.3	1.8	24.4	45.8	
2nd cut	1617	17.5	29.7	52.1	**2.3***	**25.4***	48.5	
3rd cut	1461	**18.8***	**28.6***	**50.6***	1.9	25.1*	**52.4***	
Trial Mean	1849	21.0 17.5	29.5 29.5	52.4 52.4	48.7 2.1	2.1 25.9	25.9 48.7	25.9
TK p<0.10	***	***	***	***	***	***	***	

*Variables with an asterisk indicate that it was not significantly different than the top performer in column (in **bold**).
*** Parameter was significant with a Tukey-Kramer level of significance less than 0.10.

By separating the data across site-years, we can see many differences in yield and quality (Table 9). Yields were highest at Shelburne Farms in 2012 and 2013. Crude protein levels were highest at Butterworks Farm in 2013. The ADF content was lowest at Butterworks Farm in 2013 and 2014, while NDF was lowest at Butterworks Farm in 2012—which was also the highest starch level. Non-fiber carbohydrate levels were highest at Butterworks in 2014 and digestible NDF was highest at Shelburne Farms in 2012. These differences are outlined more in the yearly reports included in the appendix.

Table 9. Yield and quality of forages treated with Nutrient Dense Sprays, reported by site-year.

	Yield	CP	ADF	NDF	Starch	NFC	NDFD	
	lbs ac^{-1}	%	%	%	%	%	% of NDF	
2012BW	2058	18.4	29.4	**45.8***	**3.4***		44.9	
2012SF	**2298***	16.9	32.1	54.4	2.3		**61.3***	
2013BW	1800	**20.5***	**26.7***	53.3	1.8	26.7	37.9	
2013SF	2231*	14.6	31.1	65.7	0.7	20.5	41.3	
2014BW	1253	18.2	27.5*	46.8*	2.6	**29.4***	57.2	
2014SF	1894	15.9	31.1	56.0	1.2	23.3	50.8	
Trial Mean	1849	17.5 21.0	29.5 29.5	52.4 52.4	2.1 48.7	25.9 48.7	25.9 48.7	25.9
TK p< 0.10	***	***	***	***	***	***	***	

*Variables with an asterisk indicate that it was not significantly different than the top performer in column (in **bold**).
*** Parameter was significant with a Tukey-Kramer level of significance less than 0.10.

Impact of Nutrient Dense Sprays on Fatty Acid Concentrations

We analyzed over 542 forage samples to determine the fatty acid profile and concentration for this study. Overall, there were no interactions of the treatments by cut, and only two interactions of the treatments by environment (for concentration of mono-unsaturated fatty acids (MUFA) (Figure 1) and saturated fatty acids (Figure 2). Interestingly the forage FA concentrations parameters showed little response NDS treatments with the exception of BF 2012 where the All, Potassium, Rejuvenate, and PhotoMag treatments increased MUFA and SFA concentrations significantly. It is unclear why the NDS treatments resulted in a positive response in this year only and why the levels were so much higher compared to other site-years. In 2012, below average precipitation and above average temperatures may have been a contributor to this response. Since only two interactions were observed in the analysis, the data was analyzed across site-years.

There were no significant differences in forage fatty acids (FA) based on the NDS treatments (Table 10). Only the concentration of saturated fatty acids (SFA) and MUFAs were significantly different by treatment; however these dependent variables also had a treatment by environment effect. The level of Omega-3 FAs did not differ among treatments. Overall, we were surprised to not see an effect from the NDS treatments. Potentially, the reasons could be that the sites chosen for this study were already sufficiently high in nutrients and therefore additional applications did not make a difference, or perhaps, the NDS washed off the foliage before they were able to be taken up by the plant, or the NDS did not provide sufficient nutrients to make a difference in yield, quality, or fatty acid content.

Table 10. Fatty acid profile (%) and concentration (mg/g) of Nutrient Dense Sprays on forages.

	All	Control	MicroPak	Phosphorus	PhotoMag	Potassium	Rejuvenate	Trial mean	TK p<0.10
Profile C16	21.1	20.9	20.5	20.9	21.1	21.0	20.8	20.9	NS
Conc C 16	4.1	4.3	4.1	4.1	4.1	4.2	4.1	4.1	NS
Profile C 18:2	19.3	19.9	19.9	19.2	19.2	19.0	19.5	19.4	NS
Conc C 18:2	3.9	4.3	4.1	3.9	4.0	4.0	4.1	4.1	NS
Profile C 18:3	44.2	44.1	44.2	44.9	44.5	44.9	44.6	44.5	NS
Conc C 18:3	9.1	9.5	9.2	9.3	9.3	9.6	9.4	9.3	NS
Profile SFA	29.1	28.7	28.4	28.8	29.0	28.9	28.6	28.8	NS
Conc SFA	5.8	5.9	5.6	5.6	5.9	6.1	5.8	5.8	***
Profile MUFA	4.1	4.0	4.2	4.0	4.0	4.0	4.1	4.1	NS
Conc MUFA	1.0	0.9	0.9	0.8	1.0	1.2	1.0	1.0	***
Profile PUFA	63.7	64.2	64.3	64.3	63.9	64.0	64.2	64.1	NS
Conc PUFA	12.9	13.7	13.2	13.1	13.0	13.3	13.2	13.2	NS
Profile O-3	44.3	44.1	44.2	45.0	44.6	45.0	44.7	44.5	NS
Conc O-3	9.0	9.5	9.2	9.3	9.1	9.5	9.3	9.3	NS
Profile O-6	19.4	20.0	20.0	19.3	19.3	19.1	19.6	19.5	NS
Conc O-6	4.0	4.3	4.2	4.0	4.0	4.0	4.1	4.1	NS
Conc Total FA	19.6	20.6	19.9	19.7	19.8	20.3	20.0	20.0	NS
Ratio O-6:O-3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	NS

NS – Not Significant, none of the variables were significantly different from one another.
*** Parameter was significant with a Tukey-Kramer level of significance less than 0.10.

There were many differences in fatty acid content of the forages by cut (Table 11). Overall, second and third cut had the highest SFAs and poly-unsaturated fatty acids (PUFA), while first cut had the highest level of MUFAs. Second and third cut had higher levels of Omega-3 FAs. However, the total concentration of FAs was not significantly different by cut. Essentially it appeared as though the harvest (cut) had a much stronger impact on FAs than the NDS treatments. Second and third cuts of perennial forage were likely much leafier and potentially fewer mature seed heads compared to the 1st cutting. These factors would have contributed to higher levels of Omega-3 concentrations.

Table 11. Fatty acid profile (%) and concentration (mg/g) of cuts of forage.

	1st cut	2nd cut	3rd cut	Trial mean	TK p<0.10

Profile C16	19.4	21.0	**21.8**	20.9	***
Conc C 16	3.8	4.1	**4.4**	4.1	***
Profile C 18:2	**27.0**	16.6	17.0	19.4	***
Conc C 18:2	**5.6**	3.4	3.6	4.1	***
Profile C 18:3	35.6	**48.0***	47.2*	44.5	***
Conc C 18:3	7.2	**10.2**	10.0	9.3	***
Profile SFA	27.4	28.7	**29.8**	28.8	***
Conc SFA	5.3	**6.0***	6.0*	5.8	***
Profile MUFA	**6.7**	3.2	3.1	4.1	***
Conc MUFA	**1.4**	1.0	0.6	1.0	***
Profile PUFA	62.7	**64.8***	64.3*	64.1	***
Conc PUFA	12.8	13.3*	**13.4***	13.2	***
Profile O-3	35.6	**48.1***	47.2*	44.5	***
Conc O-3	7.2	**10.0***	10.0*	9.3	***
Profile O-6	**27.1**	16.7	17.1	19.5	***
Conc O-6	**5.6**	3.5	3.6	4.1	***
Conc Total FA	19.6	20.0	20.3	20.0	NS
Ratio O-6:O-3	0.8	**0.4***	0.4*	0.5	***

*Variables with an asterisk indicate that it was not significantly different than the top performer in column (in **bold**).
NS – Not Significant, none of the variables were significantly different from one another.
*** Parameter was significant with a Tukey-Kramer level of significance less than 0.10.

There were many significant differences in fatty acid content of forage by site-year (Table 12). Butterworks Farm in 2013 had the highest Omega-3 profile, while 2014 at Butterworks Farm had the highest concentration of Omega-3 FAs. The total concentration of FAs was highest at Butterworks Farm in 2014. Overall Omega-3 FAs were highest at the Butterwork Farm location. Overall, all Butterworks had the highest levels of FAs in their forages and this may be related to the farms long term commitment to soil organic matter building and soil fertility. The baseline soil analysis for this field indicated organic matter levels over 6% and has had yearly applications of compost and manure. The farm also regularly applies micronutrients, potassium, and gypsum if needed.

Table 12. Fatty acid profile (%) and concentration (mg/g) of cuts of forage from each site-year of the trial.

	BW 2012	SF 2012	BW 2013	SF 2013	BW 2014	SF 2014	Trial mean	TK p<0.10
Profile C16	20.8	23.8	19.6	**24.0**	17.0	21.5	20.9	***
Conc C 16	**5.1***	4.0	4.1	3.3	4.8*	3.9	4.1	***
Profile C 18:2	18.9	16.7	**20.7**	20.0	20.6	18.3	19.4	***
Conc C 18:2	4.7*	2.8	4.4	2.8	**5.9***	3.4	4.1	***
Profile C 18:3	48.6	47.1	**47.4**	42.5	45.2	39.0	44.5	***
Conc C 18:3	12.2	8.1	10.2	6.0	**13.1**	7.2	9.3	***
Profile SFA	28.8	32.7*	27.2	**32.9***	22.8	30.2	28.8	***
Conc SFA	**8.2**	5.4	5.6	4.5	6.5	5.4	5.8	***
Profile MUFA	3.6	3.3	**4.5***	4.1	4.3*	4.1	4.1	***
Conc MUFA	**2.1**	0.6	0.9	0.6	1.2	0.8	1.0	***
Profile PUFA	67.8*	64.1	**68.2***	62.6	66.0	57.4	64.1	***
Conc PUFA	15.5	10.3	14.6	8.8	**19.0**	10.6	13.2	***
Profile O-3	48.7	47.1	**47.4**	42.5	45.3	39.0	44.5	***
Conc O-3	11.4	8.1	10.2	6.0	**13.1**	7.2	9.3	***
Profile O-6	19.0	16.9	20.8	20.1	**20.7**	18.4	19.5	***
Conc O-6	4.8*	2.9	4.4	2.8	**5.9***	3.4	4.1	***
Conc Total FA	24.9	17.0	21.2	14.0	**26.8**	16.8	20.0	***
Ratio O-6:O-3	**0.4***	0.4*	0.5	0.5	0.5	0.5	0.5	***

*Variables with an asterisk indicate that it was not significantly different than the top performer in column (in **bold**).
*** Parameter was significant with a Tukey-Kramer level of significance less than 0.10.

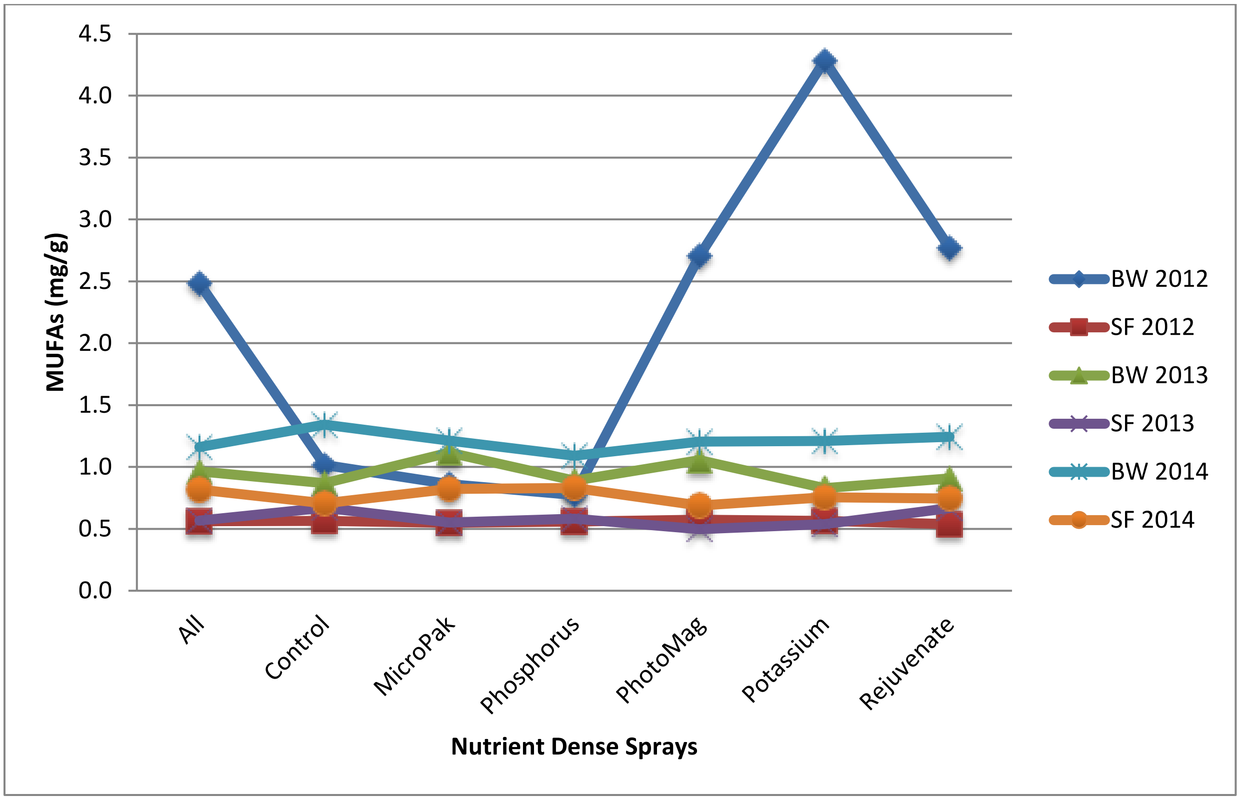

Figure 1. Concentration of MUFAs by site-year and NDS treatment.

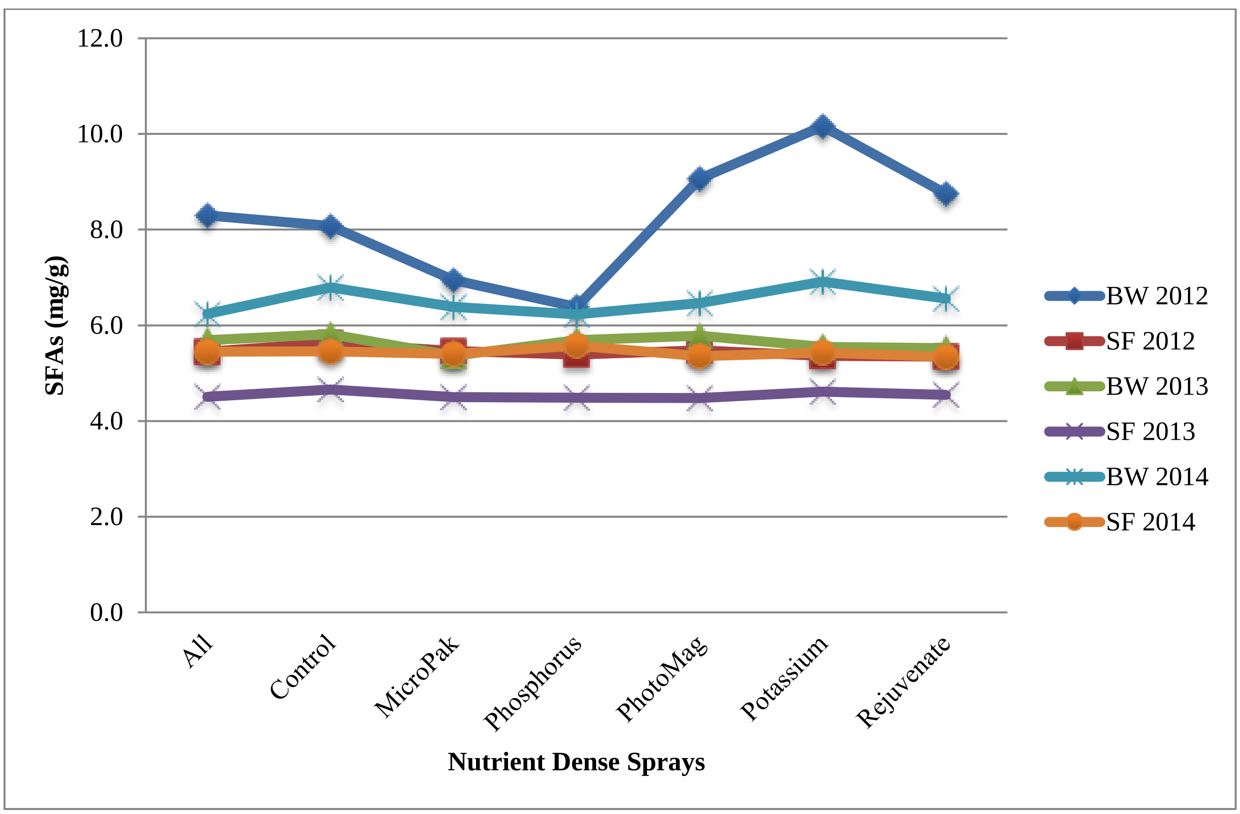

Figure 2. Concentration of SFAs by site-year and Nutrient Dense Spray treatment.

<u>Impact of Nutrient Dense Sprays on Micronutrient Concentrations of Forages</u>

Because the NDS regime did not have a significant effect on yield or quality in the first years of the study, we sent samples to Cumberland Valley Analytical Services for wet chemistry analysis of minerals. Wet chemistry is considered more accurate for detecting small differences in samples. There were no significant differences of the micronutrient levels of the 2012 forages at Butterworks Farm (Table 13). At Shelburne Farms, there were some differences detected. Interestingly, the 'All' treatment had lower Phosphorus concentrations than the Control (Table 14). More in line with what may be expected, 'All' had higher levels of Sodium and Manganese than the Control. If funds were available it would have been good to evaluate mineral content during all years of the project. It is likely that minerals would have increased over time due to repeat applications of the NDS treatments.

Table 13. Micronutrient content of forages at Butterworks Farm, VT.

Treatment	Ash %	Calcium %	Phosphorus %	Magnesium %	Potassium %	Sodium %	Iron Ppm	Manganese ppm	Zinc ppm	Copper ppm
All	9.7	1.2	0.55	0.4	2.7	0.02	132.3	44.8	44.0	15.3
Control	10.0	1.4	0.61	0.4	2.7	0.02	92.5	40.5	41.8	15.0
Trial Mean	9.9	1.3	0.58	0.4	2.7	0.02	112.4	42.6	42.9	15.1
LSD (p<0.1)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS – Not Significant, none of the variables were significantly different from one another.

Table 14 . Micronutrient content of forages at Shelburne Farms, VT.

Treatment	Ash %	Calcium %	Phosphorus %	Magnesium %	Potassium %	Sodium %	Iron Ppm	Manganese ppm	Zinc ppm	Copper ppm
All	10.3	1.1	0.45	0.3	2.8	0.032	186.6	100.1	33.6	12.9
Control	10.5	1.2	0.49	0.3	3.0	0.018	148.4	85.4	31.0	13.1
Trial Mean	10.4	1.2	0.47	0.3	2.9	0.025	166.2	92.3	32.2	13.0
LSD (p<0.1)	NS	NS	0.039	NS	NS	0.008	NS	8.56	NS	NS

NS – Not Significant, none of the variables were significantly different from one another.

Big Strip Plot Results

Because we did not see significant differences in yield or quality of the forages treated with the NDS in 2012 or 2013, we wanted to rule out that there was any contamination of treatments in the small plots (which had 3-5' alleys). Therefore, in 2014 we established large plots 11' x 55' at least 20' away from other treated areas. At Butterworks Farm, there was no significant difference in yield or quality for first or second cut (Table 15). The third cut results generally showed the opposite of what we would expect. The 'All' treatment had lower CP, higher ADF and NDF fiber content, lower Non-Fiber Content (NFC), and higher digestible NDF (NDFD). Lack of response again may be associated with the already fertile soils.

There were more differences detected at Shelburne Farms (Table 16). The Nutrient Dense Spray 'All' treatment yielded 500 lbs acre^{-1} more than the Control for first cut, 230 lbs acre^{-1}, and 792 lbs acre^{-1} more than the control for 2nd and 3rd cut, respectively, for a total of 1522 lbs acre^{-1} increase in yield from the NDS. The quality results, however, were not what we would expect. For first cut, the 'All' treatment had lower starch, higher ADF and NDF, lower NFC and higher digestible NDF. Second cut 'All' had higher CP, starch, lower ADF and NDF, and higher digestible NDF. Third cut 'All' had lower CP, lower ADF, higher NDF and lower NFC. Future research should likely allow for larger plots sizes to evaluate this yield response and lack of quality response.

Table 15. Hay yield and quality of large strip plots, Westfield, VT, 2014.

Treatment	DM yield lbs. acre^{-1}	CP %	Starch %	ADF %	NDF %	NFC %	NDFD %
1st cut All	2187	16.4	1.9	30.3	52.3	26.6	56.2
1st cut Control	2083	16.5	2.1	29.8	51.1	27.7	55.6
Trial mean	2135	16.4	2.0	30.0	51.7	27.1	55.9
LSD (p<0.10)	NS	NS	NS	NS	NS	NS	NS
2nd cut All	324	19.6	3.1	25.3	42.5	31.2	61.9
2nd cut Control	491	17.6	3.0	27.2	44.9	31.2	60.9
Trial mean	408	18.6	3.1	26.3	43.7	31.2	61.4
LSD (p<0.10)	NS	NS	NS	NS	NS	NS	NS
3rd cut All	1582	16.2	1.9	29.6	52.4	25.9	60.2
3rd cut Control	1126	20.3	1.9	25.7	42.5	30.5	54.7
Trial mean	1354	18.2	1.9	27.7	47.4	28.2	57.4
LSD (p<0.10)	NS	2.79	NS	1.87	5.33	2.03	2.06

NS – Not Significant, none of the variables were significantly different from one another.

Table 16. Hay yield and quality of large strip plots, Shelburne, VT, 2014.

Treatment	DM yield lbs. acre^{-1}	CP %	Starch %	ADF %	NDF %	NFC %	NDFD %
1st cut All	2779	13.1	0.6	31.3	65.8	20.6	41.7
1st cut Control	2279	13.7	1.4	30.2	61.0	24.1	37.2
Trial Mean	2529	13.4	1.0	30.7	63.4	22.4	39.5
LSD (p<0.10)	357	NS	0.39	1.11	3.94	2.39	3.47
2nd cut All	1788	18.4	1.4	31.4	50.8	22.7	57.7
2nd cut Control	1558	17.3	1.3	34.1	53.2	23.0	56.0
Trial Mean	1673	17.8	1.3	32.8	52.0	22.9	56.8
LSD (p<0.10)	0	0.97	0.48	1.59	2.11	NS	1.52
3rd cut All	1843	16.1	0.1	32.7	57.1	20.0	54.5
3rd cut Control	1051	19.7	-0.3	33.7	51.9	22.3	52.5
Trial Mean	1447	17.9	-0.1	33.2	54.5	21.2	53.5
LSD (p<0.10)	0	1.11	0.198	0.84	2.63	1.54	NS

NS – Not Significant, none of the variables were significantly different from one another.

There were no significant differences seen among the treatments in the big strip plots at Butterworks Farm (Table 17). This is consistent with what we saw in the small plots.

Table 17. Fatty Acid Profile (%) and Concentration (mg/g) of All and Control Treatments at Butterworks Farm, 2014.

	All	Control	Trial Mean	LSD (p<0.10)
Profile C16	16.5	16.6	16.6	NS
Conc C 16	4.5	4.7	4.6	NS
Profile C 18:2	19.3	19.2	19.2	NS
Conc C 18:2	5.5	5.5	5.5	NS
Profile C 18:3	47.4	47.2	47.3	NS
Conc C 18:3	13.3	13.5	13.4	NS
Profile SFA	22.0	22.6	22.3	NS
Conc SFA	6.1	6.4	6.3	NS
Profile MUFA	4.0	3.8	3.9	NS
Conc MUFA	1.2	1.1	1.1	NS
Profile PUFA	66.9	66.5	66.7	NS
Conc PUFA	18.8	19.1	19.0	NS
Profile O-3	47.5	47.3	47.4	NS
Conc O-3	13.3	13.6	13.4	NS

Profile O-6	19.4	19.2	19.3	NS
Conc O-6	5.6	5.5	5.5	NS
Conc Total FA	26.1	26.6	26.4	NS
Ratio O-6:O-3	0.4	0.4	0.4	NS

NS – Not Significant, none of the variables were significantly different from one another.

Interestingly, while there was no significant difference in the All vs. Control big strip plots at Butterworks Farm, there were many differences at Shelburne Farms (Table 18). The All treatment of the NDS resulted in higher profiles of C16 FA and SFAs. Otherwise, the Control had higher levels of C18:2 FA, MUFAs, PUFAs, Omega-6 FAs and Total FAs. Again a further look at NDS applications on larger research areas would be important to understand the ability of these sprays to increase fat content of forages.

Table 18. Fatty Acid Profile (%) and Concentration (mg/g) of All and Control Treatments at Shelburne Farms, 2014.

	All	Control	Trial mean	LSD (p<0.10)
Profile C16	**22.4***	21.3	21.8	0.6
Conc C 16	3.6	3.8	3.7	NS
Profile C 18:2	17.5	**20.2***	18.8	1.5
Conc C 18:2	2.9	**3.9***	3.4	0.7
Profile C 18:3	37.7	37.0	37.3	NS
Conc C 18:3	6.3	6.7	6.5	NS
Profile SFA	**31.8***	30.5	31.2	1.0
Conc SFA	5.2	5.5	5.3	NS
Profile MUFA	3.9	**4.5***	4.2	0.6
Conc MUFA	0.6	**0.9***	0.8	0.2
Profile PUFA	55.2	**57.4***	56.3	1.5
Conc PUFA	9.3	**10.7***	10.0	1.0
Profile O-3	37.7	37.1	37.4	NS
Conc O-3	6.3	6.7	6.5	NS
Profile O-6	17.5	**20.2***	18.9	1.5
Conc O-6	2.9	**4.0***	3.5	0.7
Conc Total FA	15.1	**17.1***	16.1	1.5
Ratio O-6:O-3	0.5*	0.6	0.5	0.1

NS – Not Significant, none of the variables were significantly different from one another.

There were significant differences in fatty acids by cut at Butterworks and Shelburne Farms (Table 19 and 20). In general, the first cut had higher levels of MUFAs and Omega-6 FAs, while second and third cuts had higher levels of PUFAs and Omega-3 FAs.

Table 19. Fatty Acid Profile (%) and Concentration (mg/g) of 1st, 2nd, 3rd, and 4th cut Forage at Butterworks Farm, 2014.

	1st cut	**2nd cut**	**3rd cut**	**4th cut**	**Trial Mean**	**LSD (p<0.10)**
Profile C16	16.3	**17.0***	16.9*	16.0	16.6	0.6
Conc C 16	4.5	4.8	4.6	4.5	4.6	NS
Profile C 18:2	**27.1***	15.3	17.0	17.5	19.2	2.2
Conc C 18:2	**7.8***	4.4	4.7	5.1	5.5	1.4
Profile C 18:3	37.3	**52.8***	49.7	49.5	47.3	2.8
Conc C 18:3	10.3	**15.3***	13.7	14.3	13.4	1.4
Profile SFA	22.8	21.9	22.6	22.0	22.3	NS
Conc SFA	6.3	6.3	6.2	6.3	6.3	NS
Profile MUFA	**6.6***	2.7	2.7	3.5	3.9	0.8
Conc MUFA	**1.9***	0.8	0.8	1.0	1.1	0.4
Profile PUFA	64.6	**68.2***	67.0	67.1*	66.7	1.1
Conc PUFA	18.2	19.7	18.5	19.5	19.0	NS
Profile O-3	37.3	**52.9***	49.8	49.6	47.4	2.8
Conc O-3	10.3	**15.3***	13.7	14.4*	13.4	1.4

Profile O-6	**27.3***	15.3	17.1	17.5	19.3	2.2
Conc O-6	**7.9***	4.4	4.8	5.1	5.5	1.4
Conc Total FA	26.4	26.8	25.4	26.8	26.4	NS
Ratio O-6:O-3	0.8	**0.3***	0.3*	0.4	0.4	0.1

NS – Not Significant, none of the variables were significantly different from one another.

Table 20. Fatty Acid Profile (%) and Concentration (mg/g) of 1st, 2nd, and 3rd cut Forage at Shelburne Farms 2014.

	1	2	3	Trial Mean	LSD (p<0.10)
Profile C16	19.6	**23.7***	22.1	21.8	0.7
Conc C 16	**3.9***	3.6	3.7*	3.7	0.3
Profile C 18:2	**24.5***	15.6	16.3	18.8	1.8
Conc C 18:2	**5.1***	2.4	2.8	3.4	0.8
Profile C 18:3	35.5	36.3	**40.3***	37.3	2.6
Conc C 18:3	**7.1***	5.6	6.9*	6.5	0.7
Profile SFA	27.7	**34.5***	31.4	31.2	1.2
Conc SFA	5.5	5.2	5.3	5.3	NS
Profile MUFA	**5.7***	3.8	3.0	4.2	0.7
Conc MUFA	**1.2***	0.6	0.5	0.8	0.2
Profile PUFA	**60.1***	52.0	56.7	56.3	1.8
Conc PUFA	**12.2***	8.0	9.8	10.0	1.3
Profile O-3	35.5	36.3	**40.4***	37.4	2.6
Conc O-3	**7.1***	5.6	7.0*	6.5	0.7
Profile O-6	**24.6***	15.7	16.3	18.9	1.8
Conc O-6	**5.2***	2.4	2.8	3.5	0.8
Conc Total FA	**18.9***	13.7	15.6	16.1	1.8
Ratio O-6:O-3	**0.7***	0.4	0.4	0.5	0.1

NS – Not Significant, none of the variables were significantly different from one another.

In looking at the fatty acid content of the forages in the big strip plots at Butterworks Farm, there were interactions between the treatments and cuts for the following dependent variables: C16 Profile, C16 concentration, C18:3 concentration, SFA concentration, PUFA concentration, Omega-3 concentration. The Treatment x Cut interaction for the concentration of Omega-3 Fatty Acids is shown below (Figure 3). The All treatment had higher concentration of Omega-3 FA for 1st and 2nd cut, however the Control had higher concentrations of Omega-3 FA for 3rd and 4th cuts. This may indicate that applying the NDS treatments on the earlier harvests provided more benefit than applying to later harvests of perennial forage.

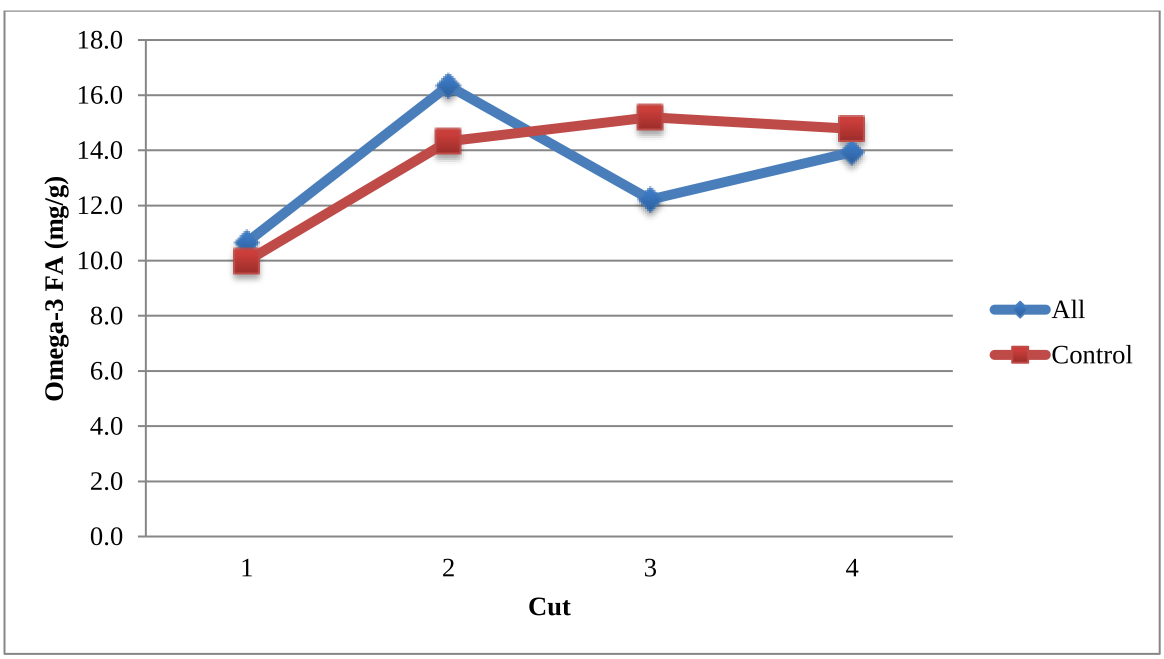

Figure 3. Treatment x Cut Interaction on the concentration of Omega-3 fatty acids in forage at Butterworks Farm, 2014.

CONCLUSION

Farmers are interested in strategies that will help them improve the yield and quality of their perennial forages. In particular, farmers would like to see forages packed with nutrients to help improve cattle health, nutrition, and ultimately reduce the purchase of off-farm concentrates. Applying foliar fertility has been identified as a means to improve nutrient density of crops. Many farms in the region have been interested in learning more about the benefits of these types of amendments. Although this experiment was conducted over 3 years, it was difficult to identify the benefits to using foliar fertility on perennial forages. The weather, baseline soil fertility, and size of plots appeared to heavily influence project results. The inability to also use wet chemistry techniques to look at mineral content of the forages may have also limited our ability to pick-up statistical differences among the treatments. Lastly, it is unclear if and what timing might be best for application of these types of amendments. As an example, in some cases first harvest responded more favorably to NDS treatments. More research should be conducted to understand the potential benefits of these types of foliar amendments.

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