EFFECTIVENESS OF RAW MILK AS A PASTURE AMMENDMENT

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ABSTRACT

The effect of raw milk, applied at the rate of 186 kg/ha, on the forage growth and nutritive value and soil quality was studied through two field and three microcosm-based experiments. During the field experiment, raw milk was sprayed on mature pastures at two Vermont farms using a paired comparison design. Twice during the growing season, forage pre- and post-grazing mass, and a wide variety of forage and soil quality parameters were measured. The raw milk had little to no effect on pasture productivity or quality at either farm. Three separate microcosm experiments were also conducted. The effect on forage above and below ground mass, tiller elongation rate, tillering rate, and other characteristics was monitored for 43 days over two cuttings. In one instance, grasses treated with raw milk tillered significantly more rapidly than grasses which did not receive the treatment ($p<0.0184$), significantly increasing above ground forage biomass. Other measured forage growth parameters were not impacted by the treatment. In other microcosm experiments, raw milk had very little impact on nitrogen mineralization and no impact soil basal respiration rate or litter decomposition rate. The results of this experiment indicate that the application of raw milk onto pasture does not significantly enhance forage production or forage and soil quality. The meager gains recorded are neither great enough to influence milk production nor consistent enough to be a reliable solution.

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CHAPTER 1

COMPREHENSIVE LITERATURE REVIEW

1.1 Introduction

Pastures are an integral component of many livestock and dairy production operations in the northeast. Over time, yield and forage quality in many pastures naturally declines; this is a major source of concern among graziers (Reeve et al., 2000). To manage this problem, graziers have traditionally resorted to either spreading large quantities of manure and other fertilizer or completely removating and reseeding. However these solutions can cause off-site environmental problems, tend be costly and time consuming, and often necessitate removing the pasture from rotation. As a result of these economic and environmental factors, many farmers are seeking alternative means of achieving high yields while using fewer costly off-farm inputs.

One option receiving increasing attention is the use of highly active biological compounds known as positive plant growth regulators, metabolic enhancers, and biostimulants (Ertani et al., 2009). These compounds, which are neither fertilizers nor pesticides, are proported to promote efficient plant nutrient uptake and enhance plant growth and development through a wide variety of mechanisms. They are typically applied in very small amounts to the soil or sprayed directly onto the plant. Consequently, they aren't usually associated with the environmental problems and high costs that typify conventional remediation options nor do they interfere with

grazing management by required the herd to avoid the pasture for a extended period.

Many graziers and researchers believe that raw milk may be an effective pasture biostimulant. Raw milk has been used as a crop amendment for centuries (Nene, 2012). It contains proteins and other compounds which are established fungicides and viricides (section 1.4.1). The amino acids present in milk proteins enhance plant tolerance to heat stress and nutrient uptake capabilities (section 1.4.3). Furthermore, many of the bacteria ubiquitous in raw milk are established beneficial, plant growth promoting soil microbes (Glick, 2012; Niranjan et al., 2005)(section 1.4.5).

Farmers and extension researchers who have applied raw milk to their pasture, at the rate of 186 kg/ha or less, have reported increased forage dry matter yield, forage quality, soil porosity, and grass brix content (section 1.3.2). Although never published nor thoroughly scientifically replicated, the results have garnered widespread community interest are greatly extolled in blogs, newsletters, and other online resourcesMcGinnis (2010).

Graziers and agricultural researchers are very interested learning more about the merits of using raw milk as a pasture amendment. Many farmers have already begun spraying raw milk onto their land. However, there is no reliable peer-reviewed literature evaluating the impact raw milk has pasture productivity or quality. Existing research pertaining to the use of raw milk as an agricultural amendment has a narrow scope and was conducted predominately in greenhouses, using annual crops. The on-farm trials that examined the impact of raw milk on pasture lacked scientific rigor and replication. These studies and trials are either not specific enough or thorough enough to warrant the widespread use of raw milk on pasture. However, because these studies have generated very promising results, additional research evaluating the use of raw milk on pasture is prudent. Farmers and researchers want to know if the claims about raw milk are true. This information will help farmers make well

informed decisions before investing their time and/or money into implementing this novel practice.

1.2 Raw Milk

1.2.1 Components of raw milk

Cow milk is a complex mixture of proteins, fat, sugars, vitamins, minerals and microorganisms. It is technically an emulsion of butterfat globules, composed almost entirely of triacylglycerols, within a water-based fluid. Fat soluble vitamins A, D, E, and K and essential fatty acids are found within this portion of the milk. Dissolved within the water-based fluid are a wide assortment of carbohydrate, proteins, and minerals. Most of the proteins are arranged in clusters called casein micelles around particles of calcium phosphate. The remaining proteins, which comprise approximately 20% of the protein in milk, by weight, are more water soluble and do not form large structures. Also dissolved in the water-based component is a wide assortment of simple one and two sugar carbohydrates, and milk salts composed of calcium, phosphate, magnesium, sodium, potassium, citrate, and chlorine (Jost, 2000).

Bacteria and other microorganisms enter milk via a wide variety of contamination sources; one can expect food grade raw milk will have approximately 10,000 colony-forming units per milliliter from 22 orders and 108 different genera (Raats et al., 2011). The quantity and variety of microorganisms present in raw milk varies widely between farms. To illustrate the variation between farms, Raats et al. (2011) studied the bacterial communities in nine bulk tanks. They recorded whether or not bacteria from certain genus were present and what proportion of the total number of bacteria they encompassed; a portion of their results are displayed in Table 1.1. The presence and quantity of bacteria differed widely from farm to farm.

Table 1.1: Frequency and concentration of raw milk bacterial communities from the bulk tanks at nine farms. Data taken from Raats et al. (2011)

Order	**Genus**	**Presence in Bulk Tanks (%)**	**Concentration *(Low Range (%))***	**Concentration *(High Range (%))***
Pseudomonadales	*Pseudomonas*	55%	2.4	57.7
Bacillales	*Bacillus*	22%	6.1	8.3
Lactobacillales	*Lactococus*	88%	1	17.4
Rhizobiales	*Rhizobiaceae*	66%	1	4

1.2.2 Raw milk as a waste product

All dairy operations generate milk that is unsalable – either because the milk contains high levels of antibiotics or colostrum, or was produced from a transition or mastitic cow. Production of this byproduct, known as waste milk, ranges from 48 to 137 pounds per cow per year (Blosser, 1979). For farmers, it can represent a large economic loss to the farm and a confounding disposal problem. Milk has biological oxygen demands (BOD) of approximately 100,000 mg/L – 100 times greater than raw sewage (Russell et al., 1998; Wendorff, 2004). When discharged into a pond, the milk can overwhelm the bacteria in the pond resulting in inefficient and highly odorous anaerobic decomposition (Bloodgood et al., 2011). If discharged into a waste-water treatment system used for treating dairy plant wash water or municipal sewage, the milk will seriously impair the plants' functioning.

Farmers have three basic options for waste milk disposal. The raw waste milk can be fed to calves; however concerns about microbial contamination and disease transmission have lead many producers to reconsider the practice. Many farmers chose to feed the waste milk to other livestock including pigs and chickens; the milk is an excellent, nutritious food supplement. However, the milk from individual cows treated with antibiotics should not be fed to livestockRussell et al. (1998). In addition, on many farms, the population of other livestock is not large enough to consume the

waste milk produced. In these situations, land application is considered the best course of action. Compared with other disposal options, spraying the solution over a pasture or agricultural field substantially reduces the environmental and health risks associated with raw milk and negates this source of agricultural pollution (Indiana Department of Environmental Management, 2002; Lehrsch and Robbins, 1996).

1.3 Biostimulants

Since the nineteenth century, huge increases in agricultural production have been realized through the use of agricultural chemicals, particularly pesticides and chemical fertilizers containing nitrogen. Through these advances, developed countries have become highly successful food producer(Syltie, 1985). However, this success is not without costs. The liberal use of fertilizers and pesticides has changed soil chemical properties and caused off-site environmental problems. The expense of off-farm inputs have become a significant part of total farm operating costs, thus decreasing farmer profit potential and increasing food cost. Moreover, the gain in yield achieved by augmenting current conventional food production practices has slowed (Syltie, 1985).

Farmers and researchers are beginning to consider the merits of incorporating biostimulants into standard agricultural practice (Jardin, 2012; Leymonie, 2012). A biostimulant may act by increasing cell metabolism, increasing chlorophyll efficiency and production, increasing antioxidant production, enhancing nutrient availability, speeding up germination and cell development, or increasing the water holding capacity of plant cells, or even the soil (Syltie, 1985). Examples of well-known biostimulants include humic acids, growth hormones, amino acids, vitamins, and enzymes (Jardin, 2012).

The value of biostimulants as a farm amendment is under debate. Many re-

searchers contend that biostimulants have the potential to revolutionary the agricultural industry (Syltie, 1985; Russo and Berlyn, 1990); many more remain highly skeptical (Karnok, 2000; Kelting et al., 1998). Regardless, it should be a major objective of modern agriculture to improve production system using practices that have minimal environmental and social drawbacks. As such, these innovative practices deserve scientific consideration.

1.3.1 Use of biostimulants in pasture

Biostimulants are particularly valuable in pastures. Their advantages, over many conventional practices, are listed below.

- Biostimulants are typically sprayed on the pasture surface without disturbing the soil, thus allowing the plants develop mature root systems and remain in continuous production. By comparison, additions of manure or fertilizers usually need to be incorporated into the soil thereby damaging soil and root structure Berlyn and Sivaramakrishnan (1996).

- Biostimulants have low recommended application rates and therefore are often less expensive than conventional fertilizers Berlyn and Sivaramakrishnan (1996).

- Animals can graze a field immediately following biostimulant application. By comparison, farmers usually keep animals off pastures following fertilizer, manure, and pesticides application (Verlinden et al., 2010)

There are a wide variety of biostimulants marketed for pasture, however only humic acids have been formally evaluated. Verlinden et al. (2010) examined the impact commercial humic substances applied either as a solution (8.3 kg humic substances ha^{-1}) or incorporated into the mineral fertilizer (3.6 to 6.4 kg humic substances ha^{-1}).

The study, which took place over two years at six experimental sites, monitored forage production rates, forage quality, and plant nutrient uptake. They found humic compounds significantly increased forage dry matter production and nutrient uptake (N, P_2O_5 *and* K_2O) during the sampling event immediately after the treatment was applied, at select sites, in the plots where the humic acids were incorporated into a mineral fertilizer. There was no consistent pattern across each of the other sites, other sampling events or other treatments. These results are particularly interesting given that humic acids have been demonstrated to have a highly significant positive impact on grass growth and nutrient uptake in greenhouse-based studies (Cooper et al., 1998; Lie et al., 1998; Nardi et al., 2002; Zhang and Ervin, 2004). The study by Verlinden et al. (2010) illustrates the difficulty of conducting biostimulant research in pastures; given the variability in forage growth and weather conditions, researchers need to collect large numbers of samples observe significant differences even when testing compounds with substancial established benefits.

The impact biostimulants have on grass has been widely examined in a greenhouse setting. Most of the studies were tailored toward the turf-grass industry. They showed that, at applications rate lower than 10 kg/ha, biostimulants significantly enhance plant chlorophyll content (Russo and Berlyn, 1990), above ground biomass (Russo and Berlyn, 1990), below ground biomass (Russo and Berlyn, 1990; Zhang and Ervin, 2004; Cooper et al., 1998), photochemical efficiency (Kauffman et al., 2007), visual quality (Mueller, 2005), disease resistance (Zhang et al., 2003), and nutrient uptake (Hafadi et al., 1997). These studies illustrate that very small quantities of biological substances can exert large impact on grass growth.

1.3.2 Milk as pasture biostimulant

There are no peer reviewed studies highlighting the effects of raw waste milk on pasture soils or plants. However, graziers have conducted numerous informal on-farm experiments investigating the effect of raw milk. In 2004, a grass-based dairy farmer and two University of Nebraska–Lincoln extension researchers conducted a simple experiment assessing the impact of applying raw waste milk, at the rate of twenty gallons per acre, to rotationally grazed pasture. They reported that, after 45 days, the dry matter yield increased 1,124 pounds/acre and the soil porosity increased 18% in the plots treated with raw milk (Gompert and Richardson, 2011). Since then, many other farmers have experimented with the practice. Subsequent farmers have reported similar increases in dry matter yield, and positive changes in porosity, brix, and forage palatability following foliar milk application (McGinnis, 2010). However, there is no numerical data available to substantiate these claims.

These observations support the theory that raw milk may be an effective pasture biostimulant. At the recommended application rate of 186 kg/ha, approximately 0.51816 mL per square meter, it is highly unlikely that the milk is supplying enough nutrients to have a discernible effect. Table 1.2 details the concentration of many soil and plant nutrients in milk and their corresponding application rate if sprayed on forage at the recommended rate. Farmers and extension researchers have speculated that the bacteria present in milk are responsible for the changes observed in the field. University of Nebraska–Lincoln, Terry Gompert, stated that:

> *"When raw milk is applied to land that has been abused, it feeds what is left of the microbes, plus it introduces microbes to the soil. The milk appears to be stimulating soil life (microbes), which enhances production in term of quality and quantity."* (Gompert and Richardson, 2011)

Others have speculated that the milk enhances plant growth through a mechanism similar to that of other commercial biostimulants. A summary of research supporting and refuting these hypotheses is provided below.

1.4 Summary of possible mechanisms

Although there is no peer-reviewed literature evaluating the effect of raw milk on pasture, many studies measured the impact of milk, milk constituents, and other dairy products, on a wide variety plant and soil variables. The results of these studies are summarized below.

1.4.1 Pesticidal properties of milk

The notion of applying raw milk as a crop amendment is centuries old. The Hindu text, *Surapala's Vrikshayurveda*, dating back to the eleventh century, lists raw milk as a means of treating downy mildews, powdery mildews, foliar rusts, and viral diseases of perennials (Nene, 2012). More recent studies have confirmed the potent fungicidal and virucidal properties of milk.

Table 1.2: Vitamin and mineral composition of milk and corresponding application rate on pasture assuming milk is sprayed at the rate of 186 kg/ha(Jost, 2000)

Components	**Concentration in Milk**		**Application Rate**	
Nitrogen, N	224	g/kg	4.31	kg/ha
Calcium, Ca	1276	mg/kg	245.94	g/ha
Iron, Fe	0.54	mg/kg	0.10	g/ha
Magnesium, Mg	139	mg/kg	26.79	g/ha
Phosphorus, P	997	mg/kg	192.16	g/ha
Potassium, K	1618	mg/kg	311.86	g/ha
Sodium, Na	525	mg/kg	101.19	g/ha
Zinc, Zn	4.07	mg/kg	0.784	g/ha
Copper, Cu	0.107	mg/kg	0.02062	g/ha
Manganese, Mn	0.043	mg/kg	0.0089	g/ha
Selenium, Se	21.4	mg/kg	4.12	g/ha

1.4.1.1 Virucidal Properties of Milk

The foliar application of milk can reduce the incidence and severity of viral infections in plants. Research has shown that spraying mature plants with diluted milk greatly reduces infection by tobacco mosaic virus in pepper, tomato, and tobacco (Denby and Wilks, 1963; Newell, 1954; Hsiech et al., 1967). Treatment with milk is also effective at reducing the incidence of infection by other viruses including leaf curl disease (Ali et al., 2001), pepper mild mottle mosaic virus, cucumber mosaic virus, bean mosaic virus, and tobacco ring spot virus (Ferguson, 2005). These studies are summarized in Table 1.3. Researchers made no attempt to determine the mechanism by which milk is impacting the viruses, however Abdelbacki et al. (2010) and Ferguson (2005) speculated that milk whey proteins inhibited viral replication.

No studies have examined the impact of raw milk on common forage viruses. However, the same mechanism that reduces the incidence and severity of viruses in certain annual crops many also reduce forage viral infection. For instance, the soil-borne wheat mosaic virus, a common pasture pathogen (McLaughlin et al., 1996), is in the same family as tomato mosaic virus and therefore has a higher likelihood of being impacted by milk spays.

The incidence of viral diseases in forage and pasture crops is relatively very high, especially among legumes, compared to other annual crops (McLaughlin et al., 1996). Viral infection can significantly reduce forage yield and photosynthetic capacity (Holmes, 1977, 1979; Jones et al., 1977). Treating the infection with milk could result in increased yields and improved forage quality. Researchers evaluating the impact of milk on annual crops noted that, in most cases a reduction in disease symptoms was associated with increased fruit yield and plant growth rate (Hare and Lucas, 1959; Ferguson, 2005; Denby and Wilks, 1963; Ali et al., 2001).

1.4.1.2 Fungicidal Properties of Milk

Milk's fungicidal properties, particularly against downy and powdery mildew, are very well established. Sudisha et al. (2011) and Kumar and Bhansali (2004) examined the impact of soaking pearl millet (*Pennisetum glaucum*) seeds in diluted raw milk for 12 to 18 hours. They found the seed pre-treatments improved resistance to downy mildew under both field and greenhouse condition among adult plants; mature plants were between 35% (Sudisha et al., 2011) and 57% (Kumar and Bhansali, 2004) less like to exhibit symptoms of downy mildew.

Different types of milk, including reconstituted skim milk, pasteurized whole milk, and raw milk, have been tested on a wide variety of plants (table 1.3). In most studies, milk is sprayed until runoff is observed in plants exhibiting the early signs of infections. Milk has been unanimously shown to reduce in the incidence and severity of powdery mildew infection to some degree in zucchini (Bettiol, 1999), grape (Crisp et al., 2006), pumpkin (Debacco, 2011; Ferrandino and Smith, 2007; Zatarim et al., 2005), and wheat (Drury et al., 2003). Some studies have shown that milk is as effective or more effective that conventional fungicides at reducing incidence and severity of powdery mildew (Debacco, 2011; Bettiol, 1999). When the fungicidal properties of different types of milk are compared, raw milk more effective than pasteurized or dried (Zatarim et al., 2005) and whole milk is more effective than skim milk (Ferrandino and Smith, 2007).

There are several hypotheses regarding the mechanism by which milk reduces fungal infections. Several components of milk are capable of damaging the proteins in the microorganisms. Studies have demonstrated that when sulfur-rich amino acids, namely methionine, and the vitamin riboflavin, are exposed to the ultraviolet radiation in sunlight they produce free radicals that are biocidal to various plant pathogenic

fungi and bacteria (Jordan et al., 1992; Tzeng et al., 1989; Crisp et al., 2006). Crisp et al. (2006) discovered that free radials targeted and damaged the fungal hyphae but had no impact on fungal conidia, indicating that a second mechanism was simultaneously at work. Ravensburg (2005) showed that the lactoperoxidase enzyme, found in high concentration in milk, forms reactive oxygen molecules under certain conditions; the free radicals oxidize protein and inhibit other important metabolic processes in a bacteria and fungi. The author found lactoperoxidase sprays reduced powdery mildew, botrytis, fusarium, vierticullium, *Spaerotheca spp.*, *Erisyphee spp.*, *Leveilulla spp.* and *Microspaera spp.* infection. Lactoferrin, an antimicrobial component of milk, will also binds to fungal membranes, altering their permeability and disrupting their osmotic balance (Crisp et al., 2006).

Several compounds in milk induce systematic resistance in plants against pathogenic fungi. Foliar spays containing phosphate and potassium salts directly induce systemic resistance, most likely by supplying key nutrients to the plants (Mucharromah and Kuc, 1991; Reuveni et al., 1995, 1997). Amino acids in milk are absorbed through the leaves where they behave like endogenous plant hormones like cytokinin and auxin; numerous studies have shown that the amino acids boost the plants immune system response (Bettiol, 1999; Kumar and Bhansali, 2004; Sudisha et al., 2011). In addition, the milk indirectly affects pathogens by inducing host resistance and stimulating antagonistic microorganisms on leaf surfaces. Stadnik and Bettiol (2001) observed that milk increases the population of microorganisms antagonistic to pathogens on leaf surfaces.

Forages are heavily impacted by disease pressure, particularly fungal pressure. Berkenkamp (1974) estimated that grass and forage diseases causes a 6-7 percent reduction in forage production in Alberta, Canada. Other researchers found that crown rust causes a 37% decrease in forage yield in tall fescue (*Festuca arundinacea*

Schreb) (Armour et al., 1973) and a 30% reduction in yield of perennial ryegrass (*Lolium perenne*) (Lancashire and Latch, 1966) in certain areas. Reducing the disease pressure in forage may increase yield and quality. Other studies have found that, by suppressing fungal pathogens, milk treatment improves plant growth rate, and harvestable yield. Among tomato seedlings sprayed with a 10% solution of skim milk before transplanting, fruit yield increased 54% compared the control; the increase in yield was accompanied by a 33% reduction in infection by leaf curl disease. Spraying milk on mature pumpkin plants increased marketable yield of pumpkins 27% the over the unsprayed control plots while also reducing the symptoms of powdery mildew 50-70% (Ferrandino and Smith, 2007).

1.4.2 Insecticidal properties of milk

There is some evidence that foliar applications of raw milk affect insect populations. Graziers who have spayed milk on their pastures reported that the treatment caused a reduction in grasshopper population (Gompert and Richardson, 2011). Ali et al. (2001) found that tomato seedlings treated with 10% skimmed milk solution before transplanting had a 50% smaller insect populations compared to the control.

1.4.3 Amino acids improve stress and nutrient uptake

Milk contains 3.5% protein, by weight. When sprayed on pastures at the recommended application rate, this equates to 6.7 kg of protein per hectare. In the presence of ultraviolet light from sunlight, the proteins undergo hydrolysis, breaking down into free amino acids and polypeptides (Gilmore and Dimick, 1979). These compounds can be readily absorbed and translocated by plant tissues (Stiegler et al., 2009; Makela et al., 1996). Inside the plant, they exhibit auxin-like activity and have been known to increase plant tolerance to abiotic stress.

Table 1.3: Summary of results from studies evaluating the pesticidal properties of milk

Type of milk product	Method of Application	Plant	Application Rate	Study Location	Effect	Reference
Skim Milk (10% Dilution)	Foliar Spray	Tomato	Sprayed to runoff	Field	Treatment reduced insect vector population causing leaf curl disease	Ali et al. 2001
Raw Milk (Multiple Levels of Dilutions)	Foliar Spray	Zucchini squash	Sprayed to runoff	Greenhouse	Milk was more effective than the conventional fungicides at reducing incidence and severity of powdery mildew	Bettiol 1999
Powdered Milk (15-30 % Dilution)	Foliar Spray	Grape	Sprayed to runoff	Greenhouse	Treatment significantly reduced the severity of powdery mildew	Crisp et al. 2006
Powdered Milk (40% Dilution)	Foliar Spray	Pumpkin	Sprayed to runoff	Field and Green-house	Treatment was as effective as the chemical control in suppressing powdery mildew	Debacco 2011
Pasteurized Milk (50% Dilution)	Foliar Spray	Wheat	–	Greenhouse	Treatment significantly reduced the severity of powdery mildew	Drury et al. 2003
Raw Milk (50% Dilution)	Seed Pretreatment	Pearl Millet	Soaked for 18h	Field	Treatment significantly reducing incidence of powdery mildew on adult plants	Kumar and Bhansali 2004
Pasteurized Milk (50% Dilution)	Foliar Spray	Pumpkins	Sprayed to runoff	Field	Treatment was 50–70% as effective in reducing foliar symptoms of powdery mildew as the chemical control.	Ferrandino and Smith 2007
Dry Skim Milk	Foliar spay	Tomato	Sprayed to runoff	Field	Treatment reduced symptoms of tobacco mosaic virus 41% compared to controls	Denby and Wilks 1963
Dry Skim Milk	Foliar Spray and Dip	Pepper, Tomato, and Tobacco	Sprayed transplants to runoff	Field	Treatment prevent highly significantly reduced spread of tobacco mosaic virus	Hare and Lucas 1959
Raw Milk (30% Dilution)	Seed Pretreatment	Pearl Millet	Soaked for 12h	Field	Treatment improved seedling vigor, induced resistance to disease, improved crop production	Sudisha et al. 2011
Multiple forms of Milk	Foliar Spray	Pumpkin	Sprayed to runoff	Field	Treatments were highly effective at controlling powdery mildew	Zatarim et al. 2005

Studies evaluating the impact of milk on foliar diseases have hypothesized that the amino acids in milk boost the plant immune system through induced resistance (Bettiol, 1999; Kumar and Bhansali, 2004). Sudisha et al. (2011) tested the theory by monitoring the impact of both raw milk and five common amino acids (L-glutamic acid, L-isoleucine, L-lysine, L-phenylalanine and L-proline) in raw milk on the physiological and biochemical defense responses of peal millet. The authors soaked the plant seeds in diluted amino acids solutions (25 mM) for 24 hours before planting. They found seed pre-treatments with the amino acids enhanced seed germination and seedling vigor as much as or more than raw milk and significantly more than the sterile water controls. In the field, the greatest protection against downy mildew was recorded in plants grown from seeds treated with the amino acids L-phenylalanine, L-isoleucine and L-proline. The seed treatment with amino acids also enhanced the vegetative and reproductive growth of pearl millet compared to the control. The authors found that the amino acids significantly increased the concentration of the defense related enzymes phenylalanine ammonia lyase, peroxidase and b-1,3-glucanase.

No studies have examined the impact of hydrolyzed milk proteins on plant growth, however commercial products, containing a wide variety of amino acids, have been studied. Kauffman et al. (2007) sprayed a bio-fertilizer composed primarily of amino acids (Macro-Sorb Foliar) onto the leaves of perennial ryegrass. They reported that plants treated with the amino acid solution exhibited 95% better photochemical efficiency and 65% better membrane themostability when exposed high air temperature (temperatures above 36 degrees). Carolina et al. (2009) applied a solution composed of amino acids and some humic acids to soil pots and monitored the development of reactive oxygen species (indicators of stress) in maize and soybean plants under well-watered and drought conditions. They found that the amino acid biostimulant had no impact on plant stress tolerance under either the well-watered or water stressed

conditions. Ertani et al. (2009) and Schiavon et al. (2008) added minute quantities of protein hydrolyze from alfalfa and meat flour to the hydroponic solution feeding maize (*Zea mays*). They found that even the smallest additions of amino acids caused significant increases in root and leaf growth (Ertani et al., 2009) and plant growth and leaf sugar accumulation (Schiavon et al., 2008).

Many studies have examined the impact of individual amino acids present in milk on a wide variety of plants. See table 1.4 for a list of the mean concentrations of each amino acid in raw milk and a list of the studies which evaluated their impacts. Spraying a dilute proline solution onto the leaves of pearl millet (*Pennisetum glaucum*) and green bean seedlings (*Phaseolus vulgaris*) enhanced plant vegetative and productive

Table 1.4: Mean concentrations of each amino acid in raw milk corresponding studies evaluating their impact on plants (Jost, 2000)

Amino acid	**Concentration in Milk (per 100 g)**	**Studies Examining the Impact**
Amino Acid Mixtures		Schiavon et al. (2008); Ertani et al. (2009); Carolina et al. (2009); Kauffman et al. (2007)
Tryptophan	0.075 g	Rao et al. (2012)
Threonine	0.143 g	
Isoleucine	0.165 g	
Leucine	0.265 g	
Lysine	0.140 g	Sudisha et al. (2011)
Methionine	0.075 g	
Cystine	0.017 g	
Phenylalanine	0.147 g	Sudisha et al. (2011)
Tyrosine	0.152 g	
Valine	0.192 g	
Arginine	0.075 g	
Histidine	0.075 g	Rana and Rai (1996)
Alanine	0.103 g	Thakur and Rai (1985)
Aspartic acid	0.237 g	
Glutamic acid	0.648 g	
Glycine	0.075 g	Rana and Rai (1996); Nasholm et al. (2001)
Proline	0.342 g	Sudisha et al. (2011); Raj et al. (2004); Rana and Rai (1996); Thakur and Rai (1985)
Serine	0.107 g	Thakur and Rai (1985)

growth (Raj et al., 2004) and calcium uptake (Rana and Rai, 1996), respectively. Rana and Rai (1996) also showed that the application of exogenous histidine, glutamine, methionine and glycine to green beans also promoted calcium uptake. Rao et al. (2012) sprayed a dilute (15 ppm) L-Tryptophan solution onto the leaves of drought stressed maize plants; the treatment significantly increased leaf relative water content, membrane stability, chlorophyll and potassium content. Thakur and Rai (1985) observed that the application of exogenous proline, alaine, and serine delayed maize wilting under drought conditions. A fairly comprehensive review of the studies evaluating the role of amino acids in plant stress responses was written by Rai (2002).

In every study in which the amino acids were applied directly to the plant or seed, the authors observed a significant positive impact. Carolina et al. (2009) did not note any significant impact when the amino acid solution to the soil. This may indicate that amino acids are only effective biostimulants when sprayed directly onto plants. The results of these studies have lead researchers to two different conclusions regarding the amino acid mode of action. Sudisha et al. (2011) and Raj et al. (2004) concluded that the amino acids induce a systematic resistance within the plant against pathogens; the biochemistry behind this phenomenon is described in detail by Hammerschmidt (1999). Other studies have concluded that amino acids elicit an auxin-like and giberellin-like activity which promotes defense responses against biotic and abiotic stress (Kauffman et al., 2007; Rao et al., 2012; Thakur and Rai, 1985), and regulates certain nutrient uptake pathways promoting more rapid nitrogen assimilation and nitrogen use efficiency in plants (Ertani et al., 2011; Schiavon et al., 2008; Rana and Rai, 1996).

Heat and water stress impede forage growth, particularly in the warm summer months. Fungal and viral diseases (described in section 1.4.1) also inhibit optimal production. Amino acid based supplements have been show to diminish the impact

these common pasture problems. As raw milk is rich in many of the same beneficial amino acids studied in the literature cited above, milk may also have a positive influence on forage production.

1.4.4 Effect on soil physical properties

Although there is very little research into the application of raw milk to soil, many studies have examined the influence of milk constituents on the soil, most notably, whey. Whey is a by-product of the cheese-making process composed of the water and milk solids that remain after the butterfat and milk proteins are removed. Although it can be used to create other salable foods, it is more commonly fed to livestock or poultry or disposed of as a waste product. The sheer volume produced and the watery nature of the liquid, make transportation and evaporation of the whey cost prohibitive. On most farms, the liquid is either fed to livestock or sprayed across agricultural fields.

Although land application was developed as a low cost, environmentally friendly waste disposal option, it may also be a valuable tool to improve pasture productivity. An overview of the studies evaluating the effects of whey on crops and soils is presented in Table 1.5. In summary, whey promotes soil aggregation, and increases aggregate stability, bacterial and fungal growth, and soil solution acidity (Lehrsch and Robbins, 1994). Consequently, whey application is associated with reduced soil erosion and increased plant growth (Peterson et al., 1979; Lehrsch et al., 2008). Improvements are especially pronounced in the western United States where soils have higher pH and sodium concentration; the addition of the acidic whey can reduce soil pH and encourage leaching of exchangeable Na (Lehrsch and Robbins, 1996). In these studies whey is applied at a high enough rate to act as both a fertilizer and soil conditioner.

Studies evaluating the impact of whey on soil chemical properties applied whey at

concentrations high enough for the whey provided either substantial soil nutrients or act as an effective soil conditioner. No study has assessed the milk of small quantities of whey on soil biogeochemical processes.

Table 1.5: Summary of results from studies characterizing the effects of whey application on soils and crops.

Property	**Rate**	**Soil Type**	**Results**	**Reference**
Soil hydraulic conductivity	200 - 800 t/ha	Sodic	Increased soil hydraulic conductivity at rates less than 400 t/ha; decreased soil hydraulic conductive at rates greater than 800 t/ha	Lehrsch and Robbins 1996
Aggregate Size	1% of soil mass	Loam	Greater mean aggregate diameter	Sonnleitner et al. 2003
Water holding capacity	1% of soil mass	Loam	No effect	Sonnleitner et al. 2003
Microbial Biomass	1% of soil mass	Loam	Increased fungal growth	Sonnleitner et al. 2003
Erosion	2.4 L/m	Silt Loam	Reduced sediment losses 75%	Lehrsch et al. 2008
Aggregate Stability	2.4 L/m	Silt Loam	Increased aggregate stability 25%	Lehrsch et al. 2008
Corn Yield and Quality	4-32 inches	Silt Loam	Increased over a four year period following whey applications	Peterson et al. 1979
Subsurface Soil P and K	4-32 inches	Silt Loam	Increase in nutrient concentration to abundant levels under all applications	Peterson et al. 1979
Corn Yield	102 mm	Silt Loam	Significant increase in yield lasting three years after application	Watson et al. 2011
Hay Yield	102 mm	Silt Loam	Large increased in yields of legume / grass mix one year after application	Watson et al. 2011
Infiltration Rate	102 mm	Silt Loam	Fourfold increase in infiltration following whey application	Watson et al. 2011
Infiltration Rate	202 - 808 Mg/ha	Sodic Silt Loam	Infiltration rate decreased with increasing whey application	Lehrsch and Robbins 1994

1.4.5 Inoculation with beneficial microbes

Raw milk has approximately 10,000 colony-forming units per milliliter representing 22 orders and 108 different genera (Raats et al., 2011). Several species of bacteria are established plant-growth promoting rhizobacteria (PGPR). These microorganisms colonize the soil surrounding the plant roots and enhance plant growth through a wide variety of mechanisms, many of which have not been well characterized. Broadly, plant growth promotion by the bacteria is achieved through phytostimulation, biofertilization, or bio-control of plant pathogens.

Nautiyal et al. (2005) studied the effect of select bacteria, isolated from raw milk, on the growth of pearl millet (*Pennisetum glaucum*). They screened 600 bacteria isolated from cow milk for their ability to inhibit the growth of pathogenic fungi and promote plant growth. Of the original bacteria, 150 strains were found to have bio-control abilities, and 50 were found to both suppress pathogens and promote plant growth. From this group, three bacteria were selected based on their ability to tolerate abiotic stress; the isolates were identified as *B. lentimorbus*, *B. subtilis*, and *B. lentimorbus*. Nautiyal et al. (2005) applied a solution containing the consortium of the 3 bacterial strains to soil and monitored its impact on pearl millet growth though a greenhouse and field study. During greenhouse studies, the authors observed that the plants grown in inoculated soil had significantly better germination rates and vigor. In field trials, the treatment of sugarcane with the consortium lead to significantly lower mortality rates, and significantly greater plant height, number of tillers, and cane girth when compared with the control. The gains achieved were not trivial; the treatment improved germination more than 30% and cane yield more than 20% as all experimental locations.

The study by Nautiyal et al. (2005) is the only one to measure the impact of

bacteria, derived directly from raw milk, on plant growth. However, many other established PGPR may also be found in raw milk. It is difficult to make broad assertions regarding the presence and concentration of specific bacteria in milk; the amount and variety of bacteria differs substantially from farm to farm (section 1.2.1). Table 1.1 clearly illustrates this variability. In addition, many of the bacteria in raw milk are not well characterized, particularly to the species level. Nonetheless, there are many studies documenting the plant growth-promoting capabilities of certain strains of bacteria they are likely in raw milk.

Lactobacillus spp., a strain of lactic acid bacteria, are one of the most common strains of bacteria in raw milk (Raats et al., 2011). They have been shown to accelerate the decomposition of organic waste. Higa and Kinjo (2000) examined if lactic acid bacteria could increase the rate of nutrient cycling and soil humus formation. Through three separate greenhouse experiments they inoculated non-sterile soil with cultures containing predominately *Lactobacillus spp.* They recorded significantly higher soil humus content and plant growth rate in pots amended with the *Lactobacillus culture.* The authors hypothesized that the bacteria increased the rate of nutrient solubilization and mineralization, thus increasing nutrient availability. Primavesi (1994) had performed a similar experiment wherein soil supporting bean and onion plants was inoculated with consortiums of organisms largely composed of *Lactobaillus spp.* They found that soil with the microograisms had higher bean and onion yields compaed to the control. The lactic acid produced by lactic acid bacteria has been shown to control plant pathogens with agricultural soil. Inoculates containing *Lactobacillus spp.* exhibt a lower incidence and severity of root-knot nematodes (Takei et al., 2008), *Fusarium oxysporum* (Hamed et al., 2011), and bacterial soft rot (*Pectobacterium carotovorum*) (Rahman et al., 2012). Interestingly, four strains of *Lactobacillus spp.* have been shown to significantly inhibit the growth of white clover plants through

the production of antimicrobial metabolites (Omer et al., 2010).

Bacillus subtilis is the predominant mesophilic spore-forming species in raw milk Scheldeman et al. (2005). Inoculating soil with *B. subtilis* often increases crop yields. The effect of *B. subtilis* on sugar cane growth was described above (Nautiyal et al., 2005). Turner and Backman (1991) achieved a 24% greater peanut yield, and improved germination, rhizobium nodulation, and root growth by inoculating the soil of potted plants with *B. subtilis.* The authors attributed the increase in growth to s plant-growth promoting substances, such as gibberellins and indole acetic acid, synthesized by the bacteria. Soil inoculates containing strains of *B. subtilis* increased plant growth up to 40% in oats and 48% in carrots in a study by Merriman et al. (1974). Almaghrabi et al. (2013) examined the impact of six plant growth promoting bacteria present in raw, including *B. subtilis* on tomato growth. They recorded significantly increased plant dry weight, plant height, and plant yields from soil inoculated with *B. subtilis*. As a result of these benefits, *B. subtilis* is commonly applied as a seed dressing in the USA and Germany for its ability to control soil-borne diseases (Niranjan et al., 2005).

A wide variety of bacteria are capable of transforming insoluble phosphorus to soluble forms by acidification, chelation, and exchange reactions (Delvasto et al., 2006). These microbes are broadly groups as phosphate-solubilizing microbes. They include strains of stains of *Bacillus*, *Enterobacter*, *Rhizobium, Flavobacterium* and *Pseudomonas,* found in raw milk (Chang and Yang, 2009). The inoculation of soil with these bacteria has significantly increase shoot and root elongations and crop yield in a wide variety of crops (Rodriguez and Fraga, 1999).

Agricultural research has discovered many additional PGPR. Kloepper et al. (1989) Parr (1994) and Lucy et al. (2004) provide a thorough summary of the study's results. Both PGPR and raw milk bacteria are incredibly diverse. For example, there

are currently 191 described *Pseudomonas spp.* Milk research has shown that bacteria within this genus are present in over 50% of raw milk samples and can comprise 2.4 and 57% of the total number of bacteriain raw milk . At the same time, *Pseudomonas spp.* have been shown to positivily influence plant growth in at least 34 different studies Lucy et al. (2004). Yet, because the specific species of *Pseudomonas* present in milk have never been classified, there is no way to determining definitively if the bacteria in raw milk will have the same impact on plant growth as the bacteria studied in PGPR research. Nonetheless, these studies illustrate that small additions of certain bacteria can have significant growth promoting abilities, and that many of these bacteria are found in raw milk.

1.5 Conclusion

The effect of raw milk on plants' growth and soil biochemistry has only been formally examined in the context of viral and fungal disease control. In these applications, milk was very effective at reducing the incidence and severity of a wide range of pathogens on a variety of crops (section 1.4.1). Under many studies treatment with milk was also associated with significantly increased crop yield. It is not clear if the yield boast is solely the result of reduced disease pressure or if another factor, perhaps some biostimulatory property of milk, played a role. Regardless, it is clear that milk is a potent pesticide. It is yet to be determined if a single application of milk can also effectively control common pasture pathogens to a degree that would positively impact forage production and quality.

Because so few studies have examined raw milk as a biostimulant, this literature review also considered the impact of individual components of raw milk on plant growth and soil biochemistry. Milk proteins break down into amino acids under

exposure to ultraviolet light. The foliar application of amino acids may induce a systematic resistance, elicit an auxin-like and giberellin-like activity, and regulate certain nutrient uptake pathways in plants. The net result is often a significant increase in crop production or quality (section 1.4.3). Studies evaluating the effect of amino acids on plant growth sprayed plant leaves with a dilute amino acid solution until run-off occurred; they did not specify the quantity applied. Therefore, we cannot compare the amino acid application rate used in previous studies to amino acid application rate expected under the recommended milk application rate. Furthermore, with the exception of the study by Sudisha et al. (2011), all experiments evaluating amino acids were conducted in a greenhouse to laboratory. It is difficult to predict if changes elicited by the amino acids will be detectable in a field setting.

Whey can boast crop yields and improve a variety of soil physical properties (section 1.4.4). However, in the studies cited above whey was applied at concentrations great enough to provide either substantial soil nutrients or act as an effective soil conditioner. Raw milk, as part of this study, will be spread at an application rate many times smaller. Therefore, it is highly doubtful that raw milk could influence plant growth or soil quality as a soil conditioner or significant nutrient source.

The farmers and researchers who experimented with raw milk as a pasture amendment hypothesized that the milk "stimulated soil microbes" (Gompert and Richardson, 2011). There is substantial research to support this theory; raw milk contains many plant-growth promoting rhizobacteria (section 1.4.5). However, the concentration of different microbes is so variable, it is impossible to compare the application rate used in previous studies to PGPR application rate expected under the recommended milk application rate. Moreover, for the milk to be an effective microbial inoculate, it needs to come in contact with the soil surfaces. Given the density of many pasture swards, this could only be ensured if the milk was applied immediately

before a rainstorm.

None of the studies outline above examined the influence of the treatment on pasture – few were even conducted in a field setting. Conducting research in a pasture poses unique complications. Pastures are incredibly diverse and fluctuating ecosystems; their growth rate and quality is a function of the time of the year, weather conditions, prior grazing events, and plant communities. To obtain accurate results, the research methodology will need to be very precise and thorough.

1.6 Research Objectives

Pastures are important to the economic success of many dairy and meat production operations; it is important to provide graziers and the research community with a thorough, objective evaluation of the practice of spraying raw milk on pasture. Since there is no existing pasture research concerning raw milk application on pasture, we set about to address the following objectives:

1. Quantify the impact of raw milk, applied at the rate of 20 gallons per acre, on pasture soil health, and forage production, quality, and composition through a field study in order to assess the practical value of raw milk as a pasture biostimulant.

2. Evaluate the impact of raw milk on soil respiration, nitrogen mineralization, litter decomposition and a variety of specific forage growth parameters in order to provide basic insight into the possible mechanism of action.

Objectives one and two shall be addressed in chapters two and three, respectively.

CHAPTER 2

RAW MILK AS A PASTURE AMENDMENT

2.1 Introduction

Pastures are an integral component of many livestock and dairy production operations in the northeast. Over time, pasture yield and forage quality tends to decline; this is a major source of concern among graziers (Reeve et al., 2000). Biological compounds known as positive plant growth regulators, metabolic enhancers, and biostimulants are a possible solution (Ertani et al., 2009). These compounds, which are neither fertilizers nor pesticides, promote efficient plant nutrient uptake and enhance plant growth and development through a wide variety of mechanisms.

Anecdotal evidence indicates that raw milk may be an effective pasture biostimulant. Raw milk has been used as a crop amendment for centuries (Nene, 2012). Numerous studies have shown that the foliar application of raw milk can reduce the incidence and severity of viral and fungal diseases (Ali et al., 2001; Bettiol, 1999; Crisp et al., 2006; Drury et al., 2003; Ferrandino and Smith, 2007; Sudisha et al., 2011)). Amino acids present in milk[1] can boost plant immune system response through in-

[1]Milk contains 3.5% protein, by weight. When sprayed on pastures at the recommended application rate, this equates to 6.7 kg of protein per hectare. In the presence of ultraviolet light from sunlight, the proteins undergo hydrolysis, breaking down into free amino acids and polypeptides (Gilmore and Dimick, 1979). These compounds can be readily absorbed and translocated by plant tissues (Stiegler et al., 2009; Makela et al., 1996).

duced resistance (Bettiol, 1999; Kumar and Bhansali, 2004), elicit an auxin-like and giberellin-like activity which promotes defense response against biotic and abiotic stress (Kauffman et al., 2007; Rao et al., 2012; Thakur and Rai, 1985), and regulate certain nutrient uptake pathways promoting more rapid nitrogen assimilation and nitrogen use efficiency (Ertani et al., 2011; Schiavon et al., 2008; Rana and Rai, 1996). Furthermore, many of the bacteria ubiquitous in raw milk are plant-growth promoting soil microbes. Nautiyal et al. (2005) showed that at least four bacteria strains commonly found in raw milk, suppress plant pathogens and at least fifty promote plant growth. When a consortium of three of the most promising microbial species was applied to sugarcane, the treatment lead to significantly lower mortality rates, and significantly greater plant height, tillering rate, and cane girth when compared to the control.

An on farm demonstration in Nebraska in which raw milk was applied to pasture at the rate of 186 kg/ha or less observed a increase in forage, have reported increased forage dry matter yield, forage quality soil porosity, and grass brix content (Gompert and Richardson, 2011). Although never published in a peer review journal, the results have garnered widespread community interest and was greatly extolled in blogs, newsletters, and other internet resources .

Graziers and agricultural researchers are very interested learning more about the merits of using raw milk as a pasture amendment. Existing research pertaining on the use of raw milk as an agricultural amendment has narrow scope and was conducted predominately in greenhouses, using annual crops. However, because these studies have generated very promising results, additional research evaluating the use of raw milk on pasture is prudent. Farmers and researchers want to know if the claims about raw milk are true. This information will help others make well informed decisions before investing their time and/or money into implementing this novel practice.

The aim of this study was to assess the effect of raw milk, applied at the rate of 186 kg/ha, on pasture soil health, and forage production, quality, and composition.

2.2 Materials and Methods

2.2.1 Study Site

Field experiments were carried out in 2012 at two Vermont dairy farms in the north-eastern section of the United States of America. Applecheeck Farm (Site 1), is a diversified organic farm located in Hyde Park, Vermont. The site is a sand (Colton-Duxbury complex (CoB)) with an average organic matter content of 9.0%; the forage was dominated by *Dactylis glomerata* L. and *Poa pratensis* L. Choiniere Family Farm (Site 2), is a family run organic dairy located in Highgate, Vermont. The site is a silt loam (Binghamville silt loam (Bg)) with an average organic matter content of 9.1%; the forage was dominated by *Lolium multiflorum* L. and *Dactylis glomerata* L. Site characteristic are shown in Table on this page. Climate at both sites is considered temperate with mean air temperature of 7 °C, ranging from -9 °C in January to a high of 22°C in July. Annual mean precipation is 91 cm and falls predominately in the autumn and spring months.

Table 2.1: Site characteristics of field sites

Property	**Site 1 (Applecheeck Farm)**	**Site 2 (Choiniere Family Farm)**
Site Size	0.88 Ha	1.56 Ha
Site Slope	0^{o}	0^{o}
Grazing Period Length	8-hour	12-hour
Soil Type	Colton-Duxbury complex (CoB)	Binghamville silt loam (Bg)
Soil Texture	Loamy sand	Silt loam
Soil Organic Matter	9.0 %	9.1 %
Dominate Pasture Vegetation	*Dactylis glomerata*	*Poa pratensis*
Secondary Vegetation	*Poa pratensis*	*Dactylis glomerata*
Proportion Legumes	4.4 % of total D.M.	20.2 % of total D.M.

2.2.2 Experimental Design

Two treatments were imposed at each site: (i) foliar application of raw milk at the rate of 186 kg/ha and (ii) a control treatment with no liquid application. A paired-comparison trial, with each pair replicated six times in established paddocks, was developed at each site. Each replicate, or paddock, contained enough forage for one half-day grazing period. Replicates were divided roughly in half and contained one pair of treatments. Treatment assignment within the first replicate was randomized; subsequent assignments were alternated[2]. Size of experimental units varied between and within sites. Experiments units at Site 1 were between 400 and $809m^2$ and averaged $736m^2$ (Figure 2.1). Experimental units at Site 2 were between 890 to 1606 m^2 and averaged $1300m^2$ (Figure 2.2).

Fresh raw milk, collected on-site, was diluted 1:1 with tapwater. Early in the grazing season, milk solution was sprayed within appropriate experimental units, approximately one week after a grazing event. The solution was applied at the rate of 372 $kg\,ha^{-1}$using a Fimco 25 Gallon ATV Mounted Sprayer. Control plots received no treatment. Date of milk application is displayed in Table 2.2.

Forage and soil measurements were made on the plots twice during the summer of 2012. At Site 1, sampling occurred within five days of the first and third grazing events post treatment application. At Site 2, sampling occurred within five days of the first and second grazing events post treatment application. Exact dates are shown

[2]The layout of Site 1 necessiated a non-random assignment of the experimental units; because the exact starting and ending location of padocks was not well defined, we chose to create wide treatment swaths; this ensured that both the treatment and control plots were grazed simultanously. At site two, a similar treatment assesignment was used to create uniformity between the sites. In addition, by alternating the treatments, we reduced the tendency fo existing field-scale soil and forage trends to influence the results. See Appendix for addition information.

Figure 2.1: Site 1: Applecheck Family Farm: Two treatments were imposed at each site: (i) foliar application of raw milk at the rate of 186 kg/ha (Labeled Milk) (ii) a control treatment with no liquid application (Labeled Control). Treatments were paired within each paddock.

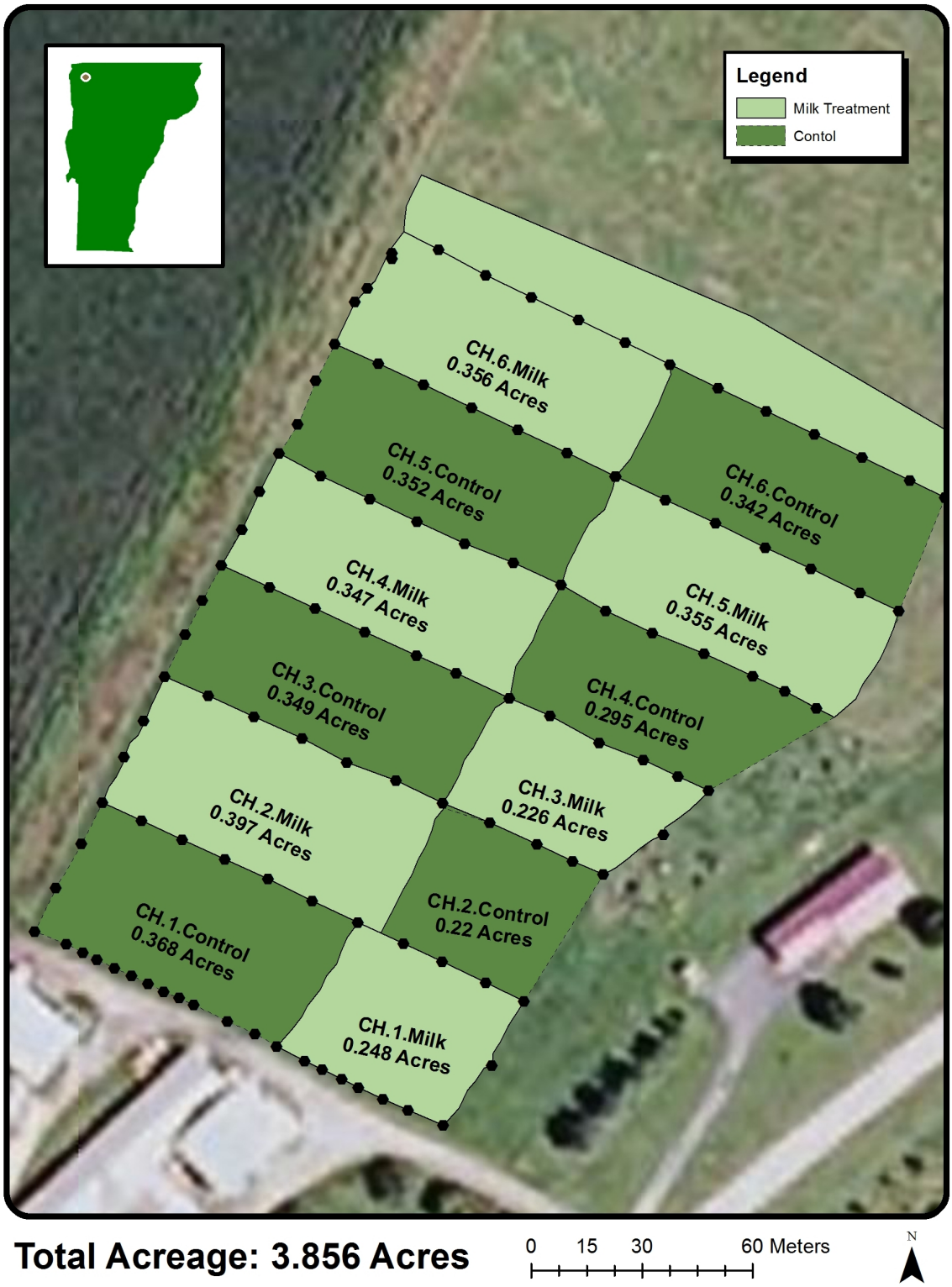

Figure 2.2: Site 2: Choinere Family Farm: Two treatments were imposed at each site: (i) foliar application of raw milk at the rate of 186 kg/ha (Labeled Milk) (ii) a control treatment with no liquid application (Labeled Control). Treatments were paired within each paddock.

in Table 2.2. Because of the replications on multiple paddocks, each sampling event took place over a four day period. This allowes sample collection to be completed at the same time per- and post- grazing.

Pregrazing forage mass was determined immediately before plots were grazed using cut samples during the first sampling event and a calibrated falling plate meter during the second sampling event. Within each experimental unit, 30 forage sample were clipped from within a 15.24 cm by 1.4 cm (0.139 m^2) quadrat (cutting height approximately 5 cm). Freshly cut samples were bagged separately, dried at $60°C$ for 72 hours, and weighed. Individual values were averaged to estimate the pregrazing mass (dry matter) of each experimental unit. Forage mass during the second sampling event and post-grazing herbage mass were measured using a falling plate meter following procedures described by Rayburn and Lozier (2003). Thirty measurements were made within each experimental unit; values were calibrated using regressions developed from 25 measurements on areas with a known mass taken at each farm during every sampling event.

The botanical composition and mineral and nutritive value of forage was measured on sample taken immediately prior to grazing. Within each experimental unit, 30 samples were cut to 5 cm height from within a $0.10m^2$ area. After the composite samples within an experimental unit were thoroughly mixed, a large sub-sample was removed for analysis. Samples were hand sorted into legume, weed, and grass fraction. Each fraction was dried at $60°C$ for 72 hours and weighed to determine

Table 2.2: Date of milk application and sample collection

Event	**Date of Events**	
	Site 1	**Site 2**
Milk Application	6/7	6/10
First Sampling Event	6/26 - 6/30	7/15 - 7/20
Second Sampling Event	8/16 - 8/21	8/12 - 8/15

estimate botanical composition on a dry matter basis. The proportion of dead material in the grass fraction was determined using digital image analysis. Sampled were photographed in a lightbox. Once acquired, images were analyzed using ImageJ 1.453 (National Institutes of Health, USA). Percent dead dry matter estimates were determined by dividing the number of brown pixels by the total number of brown and green pixels.

The grass portion was subsequently ground using a Wiley Hammer mill to pass through a 1-mm sieve. Forage quality parameters including forage protein, acid detergent fiber, neutral detergent fiber, and mineral content analyzed using infrared reflectance spectroscopy (NIR) by Dairy OneLaboratory, (Ithaca, NY).

The brix values of *Dactylis glomerata L.* and *Festuca arundinacea L.* from Site 1 and Site 2, respectively, were measured during the first sampling event. Random grab samples of 6-10 leaves were collected from 30 locations in each experimental unit. Each sample was vigorously rolled between researchers' hands for 15 seconds to form a tight ball; the sap was then extracted used a garlic press. Brix values for each batch were measured immediately using a Vee Gee Scientific STX-3 Handheld Refractometer.

Soil moisture content and electrical conductivity were measured at each site during every sampling event at 30 locations within each experimental unit using a handheld WET 2 sensor (Delta T devices, Cambridge England) . Soil samples from the uppermost 5cm were collected at 30 locations with each experimental unit, thoroughly mixed, and dried at $60°C$ for 72 hours and ground to 2mm. A sub-sample was analyzed by University of Vermont, Agricultural and Environmental Testing Lab using a Modified Morgan method for nutrients, including available phosphorus (P), potassium (K), magnesium (Mg), aluminum (Al), calcium (Ca), zinc (Zn), sulfur (S), manganese (Mn), boron (B), copper (Cu), iron (Fe), sodium (Na), cation exchange

capacity (CEC), percent calcium (%Ca), percent potassium (%K), and percent magnesium (%Mg). The pH was determined using a Mehlich buffer method with water. CEC was calculated from the %Ca, %K and %Mg. The percent organic matter (%OM) was determined by loss on ignition.

2.2.3 Data Analysis

Measurements made within each experimental unit were averaged to obtain a mean value for each experimental unit. The quanity of protein and minerals available in the forage per hectare was calculated for each experimental unit by multipling the pasture pregrazing mass by the percentage of protein/minerals in the forage. All data was analyzed using a paired t-test. All statistical calculations were performed using JMP 9.0 (SAS Institute Inc.).

2.3 Results

2.3.1 Effects of milk on herbage production, composition, and quality

For all sampling events and both sites, the application of raw milk had no statistically significant effect on herbage production (Table 2.3). Cows consumed significantly less forage from experimental units treated with milk at Site 1 during the second grazing event ($P<0.012$); however difference did not occur at Site 2 during either of the grazing events. An analysis of all of the data from both cuts at both farms reveals no statistically significant change in forage production, consumption as a result of the treatment. Forage botatanical composition did not differ between treatments during any of the sampling events (Table 2.3)

The mean concentrations of a wide variety of forage quality variables was measured on the grass fraction (Table 2.3) during each sampling event in plots with and without

Table 2.3: Mean forage production, composition and quality for Site 1 during each sampling event. Standard error (SE) and P-value given by a paired t-test analyses displayed for each farm during each sampling event.

		First Sampling Event				Second Sampling Event			
Item	**Unit**	**Control**	**Milk**	**SE**	**P-Value**	**Control**	**Milk**	**SE**	**P-Value**
Pature Pregrazing Mass	$kg\,dm\,ha^{-1}$	2511.67	2465.32	102.90	0.67	2023.19	1922.02	59.10	0.15
Pasture Post-Grazing Mass	$kg\,dm\,ha^{-1}$					1076.61	1176.96	74.41	0.24
Forage Consumption	$kg\,dm\,ha^{-1}$					946.58	745.06	52.37	**0.01**
Legume (%)	$\%\,of\,dm$	10%	9%	7%	0.40	9%	10%	1%	0.32
Weed (%)	$\%\,of\,dm$	7%	17%	2%	0.78	10%	11%	1%	0.50
Percent Dead Material	$\%\,of\,dm$	51%	52%	4%	0.48	40%	40%	2%	0.84
Acid Detergent Fiber	$\%\,of\,dm$	37.88	38.37	0.77	0.56	34.90	35.53	0.43	0.20
Neutral Detergent Fiber	$\%\,of\,dm$	58.73	58.88	1.11	0.90	53.27	54.18	1.59	0.59
Available Protein	$\%\,of\,dm$	13.70	13.82	0.64	0.86	17.47	17.27	0.70	0.79
	$kg\,dm\,ha^{-1}$	367.0	369.9	14.5	0.84	373.4	349.7	17.7	0.23
Soluble Protein	$\%\,of\,CP$	42.83	45.83	1.00	**0.03**	39.33	36.50	0.91	**0.03**
Degradable Protein	$\%\,of\,CP$	68.00	69.00	1.44	0.52	62.67	63.50	0.70	0.29
Lignin	$\%\,of\,dm$	5.33	5.82	0.42	0.30	5.85	6.37	0.39	0.25
Water Soluble Caribs	$\%\,of\,dm$	9.42	8.83	0.63	0.40	10.10	9.68	0.70	0.58
Simple Sugars	$\%\,of\,dm$	6.15	5.75	0.37	0.33	5.60	5.88	0.18	0.17
BRIX		7.70	8.56	2.80	0.57				
Phosphorus	$\%\,of\,dm$	0.33	0.33	0.01	0.42	0.37	0.35	0.02	0.54
	$kg\,dm\,ha^{-1}$	8.2	8.3	0.43	0.84	7.3	6.7	0.33	0.12
Calcium	$\%\,of\,dm$	0.58	0.68	0.02	**0.01**	0.65	0.64	0.06	0.88
	$kg\,dm\,ha^{-1}$	14.6	16.7	1.20	0.13	13.3	12.3	1.46	0.51
Potassium	$\%\,of\,dm$	2.43	2.47	0.11	0.73	2.78	2.77	0.09	0.87
	$kg\,dm\,ha^{-1}$	61.3	61.5	1.78	0.93	56.1	53.0	2.15	0.20

Table 2.4: Mean forage production, composition and quality for Site 2 during each sampling event. Standard error (SE) and P-value given by a paired t-test analyses displayed for each farm during each sampling event.

		First Sampling Event				Second Sampling Event			
Item	**Unit**	**Control**	**Milk**	**SE**	**P-Value**	**Control**	**Milk**	**SE**	**P-Value**
Pature Pregrazing Mass	$kg\,dm\,ha^{-1}$	2276.78	2417.19	75.70	0.12	2182.61	2176.45	115.43	0.96
Pasture Post-Grazing Mass	$kg\,dm\,ha^{-1}$	1384.51	1519.62	125.55	0.33	1276.80	1200.71	55.88	0.23
Forage Consumption	$kg\,dm\,ha^{-1}$	892.27	897.56	171.31	0.98	905.82	1222.15	299.40	0.34
Legume (%)	$\%\,of\,dm$	15%	16%	3%	0.68	13%	13%	2%	0.89
Weed (%)	$\%\,of\,dm$	5%	5%	1%	0.86	6%	9%	1%	**0.01**
Percent Dead Material	$\%\,of\,dm$	32%	38%	6%	0.23	47%	51%	2%	0.32
Acid Detergent Fiber	$\%\,of\,dm$	36.57	36.00	0.73	0.47	35.75	35.47	0.97	0.78
Neutral Detergent Fiber	$\%\,of\,dm$	55.98	57.70	1.41	0.28	57.27	56.47	1.57	0.63
Available Protein	$\%\,of\,dm$	14.63	14.77	0.81	0.88	16.92	16.67	0.58	0.68
	$kg\,dm\,ha^{-1}$	354.7	377.5	29.3	0.47	389.9	384.4	25.43	0.83
Soluble Protein	$\%\,of\,CP$	39.67	39.33	0.95	0.74	36.83	36.33	1.28	0.71
Degradable Protein	$\%\,of\,CP$	66.50	68.67	0.98	0.08	64.83	66.00	1.54	0.48
Lignin	$\%\,of\,dm$	5.58	4.03	0.37	**0.01**	4.00	4.45	0.36	0.26
Water Soluble Caribs	$\%\,of\,dm$	11.25	10.97	0.66	0.69	9.00	9.07	0.74	0.93
Simple Sugars	$\%\,of\,dm$	10.9	11.9	0.60	0.07	5.10	4.90	0.21	0.39
BRIX		11.61	11.27	0.41	0.45				
Phosphorus	$\%\,of\,dm$	0.32	0.33	0.02	0.73	0.37	0.37	0.01	0.73
	$kg\,dm\,ha^{-1}$	7.4	8.0	0.65	0.41	8.0	8.0	0.50	0.90
Calcium	$\%\,of\,dm$	0.59	0.55	0.03	0.29	0.50	0.55	0.07	0.55
	$kg\,dm\,ha^{-1}$	13.3	13.3	1.20	0.94	10.9	11.9	1.52	0.54
Potassium	$\%\,of\,dm$	2.74	2.73	0.15	0.98	2.79	2.74	0.22	0.82
	$kg\,dm\,ha^{-1}$	62.4	66.0	5.47	0.53	60.6	59.7	5.81	0.87

milk. The treatment had no statistically significant effect on the proportion of acid detergent fiber, neutral detergent fiber or available protein within forage samples during any sampling event.

The forage protein composition differed between treatments. During the first sampling event at Site 1, forage protein from plots treated with milk was composed of significantly more soluble protein ($P<0.026$); the opposite pattern occurred during the second cut at Site 1 ($P<0.030$). The treatment did not influence soluble protein concentration at Site 2. The percentage of degradable protein within the crude protein fraction was greater in plots treated with milk during every sampling event; an analysis of all of the data from both cuts at both farms revealed a statistically significant increase in degradable protein concentration as a result of the treatment ($P<0.0438$).

Other forage quality parameters differed as a result of the treatment. The proportion of lignin in the grass fraction of forage samples was significantly lower in the first cut taken from Site 2 ($P<0.0081$). Milk treatment had no impact on lignin at Site 1 or during the second sampling event at Site 2. Calcium concentration within forage was greater in forage treated with milk ($P<0.008$). There was no significant difference in calcium concentrations in forage during other sampling events. There was no significant difference during any sampling event for the following other parameters including water soluble carbohydrates, simple sugars, brix, phosphorous, and potassium concentrations.

2.3.2 Effects of Milk on Soil Quality

Most soil quality parameters were unaffected by the treatment. Analysis of the data from all sampling events revealed a statistically significant increase in soil organic matter concentration following the application of raw milk. When comnining all

plots and all sampling events, the concentration of organic matter in soil samples was greater ($P<0.03$) in plots treated with milk compared to the control. Other parameters including soil moisture, electrical conductivity, pH, CEC, available phosphorus, calcium, potassium and magnesium were not significantly different between treatments (Table 2.5).

Table 2.5: Mean soil quality within sites during each sampling event. Standard error (SE) and P-value given by a paired t-test analyses displayed for both sites during each sampling event.

		First Sampling Event				Second Sampling Event			
Item	**Unit**	**Control**	**Milk**	**SE**	**P-Value**	**Control**	**Milk**	**SE**	**P-Value**
Site 1:									
Soil Moisture	%	36.28	35.55	1.78	0.46	21.03	21.17	0.47	0.77
Electrical Conductivity	mS/M	55.50	55.22	2.07	0.66	76.43	76.56	2.90	0.97
Organic Matter	%	9.00	9.02	0.14	0.91				
pH		6.48	6.43	0.02	0.08				
Cation Exc. Capacity	$meg^{+}/100g$	12.98	13.42	0.25	0.15				
Available Phosphorus	mg/kg	19.17	17.30	1.42	0.25				
Potassium	mg/kg	182.33	196.67	20.10	0.51				
Magnesium	mg/kg	236.17	242.33	6.51	0.39				
Calcium	mg/kg	2110.83	2178.00	51.41	0.25				
Site 2:									
Soil Moisture	%	30.08	28.32	2.12	0.44	40.56	40.84	2.66	0.92
Electrical Conductivity	mS/M	75.08	77.79	3.33	0.45	70.49	70.03	2.98	0.88
Organic Matter	%	8.67	9.42	0.30	**0.05**	9.05	9.33	0.27	0.33
pH		6.55	6.60	0.10	0.65	6.60	6.58	0.14	0.91
Cation Exc. Capacity	$meg^{+}/100g$	13.43	13.48	0.65	0.94	13.72	13.75	0.88	0.97
Available Phosphorus	mg/kg	15.60	18.67	3.38	0.41	15.07	15.05	2.14	0.99
Potassium	mg/kg	308.00	357.17	31.10	0.17	390.67	382.67	57.75	0.90
Magnesium	mg/kg	228.33	242.83	19.37	0.49	235.17	230.83	11.51	0.72
Calcium	mg/kg	2144.00	2106.00	145.94	0.81	2147.17	2165.17	184.75	0.93

2.4 Discussion

Raw milk has been credited as a potent pasture biostimulant. Farmers have reported that a single application of raw milk, at the rate of 186 kg/ha, boasts forage yields as much as 1259 kg / ha, reduces soil porosity, and increases forage quality after a single application (Gompert and Richardson, 2011; Jehuchris et al., 2009; McGinnis, 2010). However, there is a dearth of scientific evidence regarding its effectiveness. This paper is the first comprehensive study of the impact of raw milk on pasture.

Because so little was known about the mechanism of action or net impact of raw milk, a wide variety of variables were sampled with the aim of detecting any conceivable consequence of the treatment. The data from each sampling event at each farm was analyzed separately using a paired t-test to capture both short-term and location dependent effects. The results indicated that the application of raw milk had a very influence on certain forage and soil quality parameters, at certain sites, at certain times. There were no consistent trends across sites or sampling events. Of the 135 separate statistical analyses completed, only seven were statistically significant at the 0.05 alpha level. One would expect, at this significance level, 6 to 7 Type I, false positive errors. Therefore, it is highly probably that the statistically significant results described above represent statistical anomalies.

Given that the experiment detected very few significant differences between the control and raw milk treatments, the next important consideration is whether the experimental design was powerful enough to detect true differences or did the experimental design lend itself to Type II, false negative errors. To complete the power calculations[3], the effect size was fixed at ten percent of the grand mean

[3]Power refers to the probability that your test will find a statistically significant difference when such a difference actually exists. It is generally accepted that power should be at least 0.8 or 80%.

$(0.10 * grand\,mean)$. The sample size was fixed at 12 under the assumption that the treatment effect would have occurred at both farms during one of the sampling events ($6\,experimental\,units\,per\,farm \times 2\,farms$). The statistical significance criterion used for the analysis was 0.05, as per standard practice. The result of the power calculation can be interpreted as the likelihood of detecting significant treatment effect, when such difference actually exists, assuming the treatment had an impact at both site and the treatment caused an increase or decrease over the control of at least 10%. The results are displayed in Table 2.6.

Table 2.6: Power of the experimental design to detect a difference assuming (1) the effect size was fixed at ten percent of the grand mean $(0.10 * grand\,mean)$, (2) the sample size was fixed at 12 under the assumption that the treatment effect would have occurred at both farms during one of the sampling events ($6\,experimental\,units\,per\,farm \times 2\,farms$), and (3) the statistical significance criterion used for the analysis was 0.05, as per standard practice.

Variable Type	**Variable**	**Grand Mean**	**Detectable Difference**	**Power**
Forage Production	Pre-Grazing Mass (kg/ha)	2277	227	91%
	Post - Pasture Mass (kg/ha)	1272.54	127.25	36%
	Mass Consumed (kg/ha)	1106.87	110.69	14%
Forage Quality	Acid Detergent Fiber (%DM)	36.30	3.6	100%
	Neutral Detergent Fiber (%DM)	56.56	5.7	100%
	Available Protein (%DM)	15.65	1.6	88%
	Lignin (%DM)	5.17	0.5	26%
	Simple Sugars (%DM)	5.63	0.6	39%
	Ash (%)	9.90	0.99	100%
	Crude Fat (%)	3.82	0.38	100%
	Potassium (%)	2.68	0.27	92%
	Calcium (%)	0.59	0.06	46%
	Phosphorus (%)	0.35	0.03	99%
	Brix	9.88	1.0	83%
Soil Quality	pH	6.54	0.7	100%
	Moisture (%)	31.5	3.2	63%
	Cation Exchance Capacity	13.46	1.35	95%
	Organic Matter (%)	9.08	0.9	99%
	Phosphorus (mg/kg)	16.81	1.7	14%
	Magnessium	235.94	23.59	83%
	Calcium (mg/kg)	2141	214	55%

The sampling regime established in the field experiment was very capable of detecting a change in forage growth, and forage ADF, NDF, Brix and protein concentration ($\beta > 80\%$). It is unlikely that the experiment would have detected changes in forage lignin and simple sugar content ($\beta < 80\%$). Of the key soil quality variables, the experiment would have detected a change in organic matter and pH but would have likely missed a change in phosphorus, calcium, and moisture concentration (Table 2.6). Overall, the experiment was thorough enough to conclude, with a high degree of certainty, that the treatment had no impact on forage production, important forage quality parameters (ADF, NDF, Available Protein, and BRIX), and some soil quality parameters (pH).

We can only conclude that the application of raw milk on pasture has no affect during these field experiments. There are several possible explanations. During the summer of 2012, the experimental sites received very little precipitation. Between the date the treatment was applied and the first sampling event, 2.51 and 7.41 centimeters of rain fell on Site 1 (Figure 2.4) and Site 2(Figure 2.5), respectively. Furthermore, within five days of the treatment application, Site 1 recieved 0.127 cm and Site 2 recieved 1.651 cm of rainfall. As a result, it is unlikely, particually at site 1, that the milk sprayed onto plant leaves was washed into the soil via a natural precipitation event. In addition, the droughty summer conditions also impacted the soil moisture levels. Figure 2.3 displays a 10 year average (2000-2010) and 2012 soil moisture profile from nearby research site. Soil moisture content, throughout the duration of the field experiment, was significantly lower than average. Soil fauna, under these dry conditions, would have been substantially less active and nutrient cycling would progress slowly (Coleman et al., 2004).

There is some speculation that raw milk applied to pasture "feeds what is left of the microbes, plus it introduces microbes to the soil" Gompert and Richardson

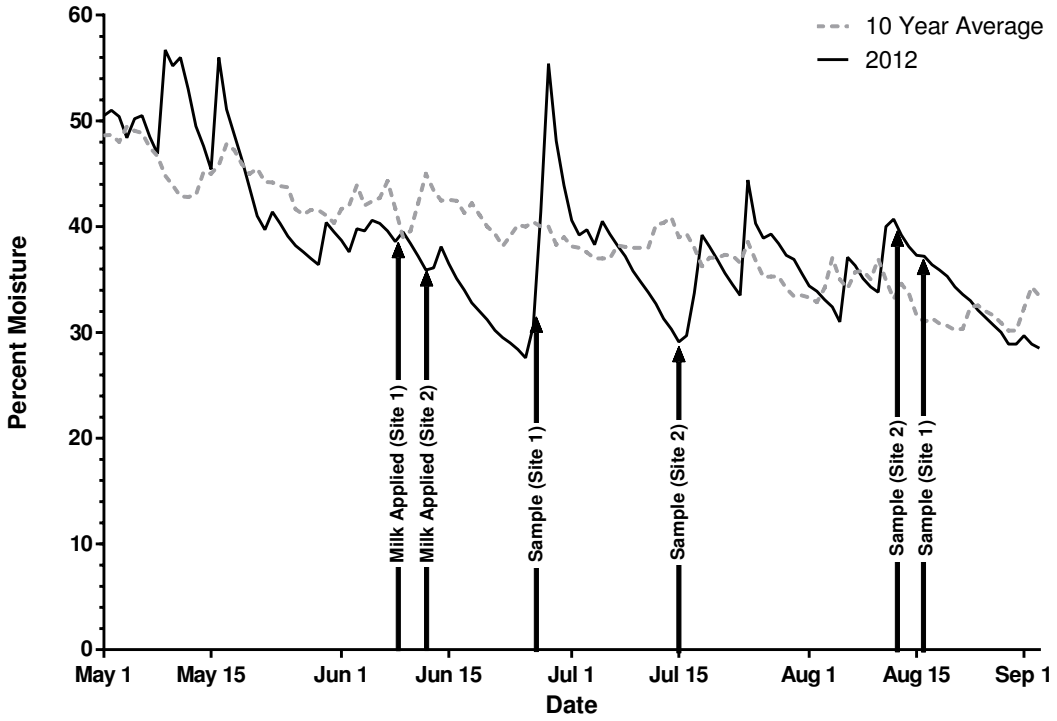

Figure 2.3: Ten year average and 2012 soil moisture at the SCAN Site at the base of Mt. Mansfield, Vermont. Data provided the Natural Resources Conservation Service National Water and Climate Center.

(2011). Although the theory has never been tested, raw milk does contain an assortment of plant growth promoting rhizobacteria (PGPR) (Nautiyal et al., 2005). These microbes may promote plant growth directly by modulating plant hormone levels or enhancing nutrient availability, or indirectly by acting as bio-control (Glick, 2012). For these processes to take effect, the raw milk needs to come in contact with the soil surface. The dry conditions may have inhibited the movement of the beneficial bacterial into the soil thereby negating the potential for milk to positively influence soil and forage parameters.

The potential of the treatment to influence the severity of pasture pathogens was indirectly evaluated by estimating the proportion of standing dead material in each

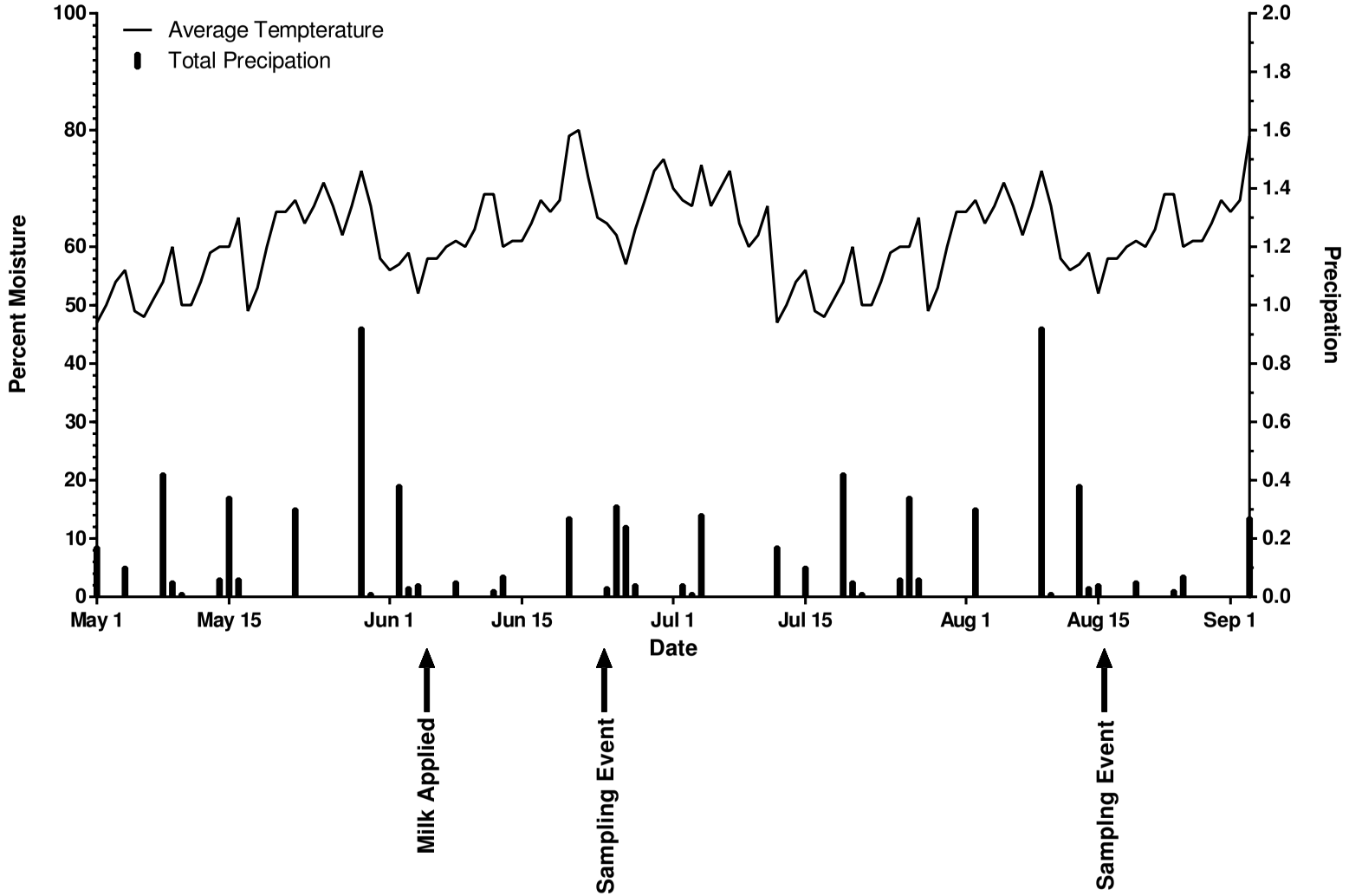

Figure 2.4: Average daily temperature and total daily precipation for Morristowm Vermont, 6 miles southwest of site Site 1. Data provided NOAA, National Climatic Data Center.

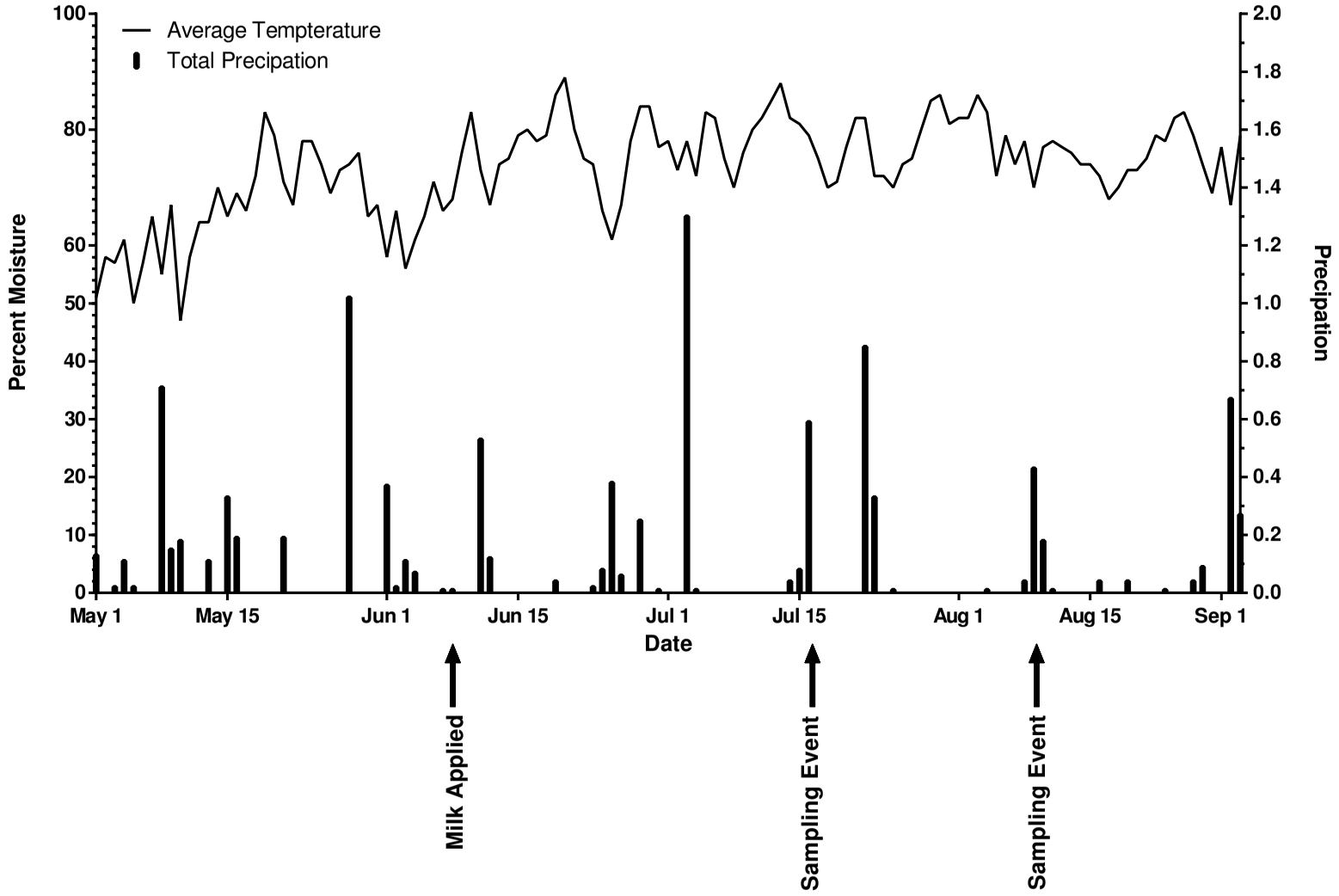

Figure 2.5: Average daily temperature and total daily precipation for Swanton Vermont, 2 miles southwest of site Site 2. Data provided NOAA, National Climatic Data Center.

experimental unit. However, under the dry conditions, a significant proportion of all the forage turned brown making it impossible to estimate the disease prevalence. Therefore, this experiment was unable to determine if the milk treatment influenced forage pathogens.

The results clearly show that milk had no effect on yeild and key forage quality parameters at either of the farms examined. However, it is important to note that the existing soil and forage quality at both sites was good. Soil organic matter concentrations, for example, at both sites, were over 9 percent. It is possible that the existing forage and soil were of too high a quality to be significantly influenced by the treatment.

Milk is rich in protein which, in the presence of sunlight, the proteins undergo hydrolysis, breaking down into free amino acids and polypeptides[4] (Gilmore and Dimick, 1979). These compounds can be readily absorbed and translocated by plant tissues (Stiegler et al., 2009; Makela et al., 1996). Inside the plant, the amino acids increase plant tolerance to heat stress and moisture deficit (Kauffman et al., 2007; Rao et al., 2012; Thakur and Rai, 1985). Despite the hot dry conditions of the summer of 2012, no significant change in forage production or quality was observed as a result of the milk treatment. It is possible that amino acids were not applied in great enough concentrations or that the effect was not detectable in a field setting.

The results of this experiment indicate that the application of raw milk onto pastures does not enhance forage production or forage and soil quality to a degree that would impact farm production. The meager gains recorded are neither great enough to influence milk production nor consistent enough to be a reliable solution. The milk had no negative effects on pasture, therefore, it is a good practice for discarding of

[4]Milk contains 3.5% protein, by weight. When spayed on pastures at the recommended application rate, this equates to 6.7 kg of protein per hectare.

waste milk. However, additional field studies under varied environmental and edaphic conditions should be conducted to confirm these results.

CHAPTER 3

THE EFFECT OF RAW MILK ON FORAGE GROWTH ATTRIBUTES AND SOIL BIOCHEMICAL PROCESSES

3.1 Introduction

Raw cow milk has been proposed as a possible low cost and effective biostimulant in pasture. During field trials, raw milk was shown to enhance forage production and forage quality and reduce soil compaction at remarkably low application rates, typically 186 kg ha^{-1}. Researchers conducting the trials hypothesized that milk stimulated soil microbes, however their hypothesis was never formally evaluated (Gompert and Richardson, 2011).

Other non-pasture studies have confirmed that small quantities of raw milk can influence soil biochemical processes. The bacteria present in raw milk, specifically lactic acid bacteria, accelerate the decomposition of organic amendments (Higa and Kinjo, 2000). Soil inoculates comprised of mainly lactic acid bacteria, increased the yield of bean and onions crops (Primavesi, 1994), mustard and radish (Higa and Kinjo, 2000), and sugarcane (Nautiyal et al., 2005), most likely by modifying the soil microbiological equilibrium thus accelerating the release of nutrients. Pearl millet grown from seeds soaked in dilute raw milk for 18 hours exhibited greater vegetative and reproductive growth compared to the control (Sudisha et al., 2011; Kumar and Bhansali, 2004). Existing soil biostimulants, applied in similar concentrations,

function in a similar manner by stimulating the rapid mineralization of nitrogen from organic materials in the soil (Chen et al., 2002, 2003).

Through a field study (Chapter 2), we examined the impact of raw milk on general forage production and soil quality parameters. In the present laboratory and greenhouse studies, a similar treatment was applied to small, uniform, soil and plant microcosms. The aim was to determine how raw milk effects soil respiration and nitrogen mineralization and specific forage growth parameters. By conducting the experiment on uniform experimental units with controlled climatic conditions, significant differences between the treatments could be more easily observed. In addition, the results will provide some basic insight into the specific mechanism by which raw milk influences soil and forage properties.

3.2 Materials and Methods

3.2.1 Materials

All three laboratory and greenhouse based studies used an Adams and Windsor loamy sand collected from the top 20 cm surface of soil in agricultural research plots in South Burlington, Vermont. The soil were sieved through a 1 cm mesh to remove organic debris and gravel. Soils were then stored no longer than three weeks before use. Soil chemical properties are displayed in Table 3.1. The fresh, unpasteurized milk used as part of the laboratory and greenhouse experiments was obtained from Family Cow Farmstand, in Burlington Vermont.

In each treatment milk was applied at the rate of $18.7\,mL\,m^{-2}$, correlating to the amount recommended in common literature.

Table 3.1: Chemical properties of soil used for all greenhouse and laboratory studies. All concentrations on dry-matter basis.

Parameter	Unit	
Soil pH (H2O)		7.3
Organic Mater	%	4.8
Cation Exchange Capacity	$mmol\,g^{-1}$	9.2
Available P	mg/kg	36.6
Potassium	mg/kg	669
Magnesium	mg/kg	167
Calcium	mg/kg	1222
Sulfur	mg/kg	17
Sodium	mg/kg	23

3.2.2 Soil Respiration

The impact of raw milk on soil respiration rates was investigated by monitoring the carbon dioxide fluxes from soil microcosm weekly for four weeks. The experiment was laid out in a complete randomized block design with two soils and two experimental treatments, each with five replicates. The treatments were: i) foliar application of raw milk at the rate of $18.7\,mL\,m^{-2}$ and (ii) a control treatment with no liquid application. To investigate the effects of the treatment on soils with differing carbon and nitrogen availability, the soils were either amended with grass leaf litter or left unamended. The organic amendments were finely ground (1 mm) and mixed through the soil at a ratio of 50:1 (soil/amendments dry wt. basis) prior to treatment application.

Five hundred grams of fresh soil (approx. 440 g dry wt.), was solely packed into cylindrical glass jars with a volume of 1892 cm^3(23 cm tall $\times$ 11 cm in diameter). To allow the biochemical processes within the soil to stabilize, microcosms sat for three weeks before treatment was applied. Over the course of the experiment, equal amounts of water were added to the soils every 2 to 3 days to maintain the soil water contents close to field capacity (between 12 and 15%, dry wt. basis). Raw milk, diluted 50 times was sprayed onto the soil surface of half of the microcosms at

concentrations equivalent to the recommend field application rate; the remaining half received deionized water.

Carbon dioxide flux rates were measured from each microcosm 7, 14, 21, and 28 day after the treatment was applied. During each sampling event, jars were sealed with a cap containing an airtight septum. Head-space samples were collected using a gas-tight syringe at three time points: 0, 90, and 180 minutes. For each time-point, 60mL of mixed head-space gas was removed from each chamber using a gas-tight syringe and transferred into 10-ml glass bottles sealed with Geo-Microbial Technologies septa. All gas samples were analyzed within 12 hours of collection. Concentrations of carbon dioxide were measured using a GC-17A gas chromatograph (Shimadzu Scientific Inc., Columbia, Maryland, USA), equipped with a Porapak-Q column electron capture detector (ECD). The instrument was specifically configured and programmed for greenhouse gas analysis. The instrument utilizes a 2 m HayeSep D and 1 m-Porapack Q column and uses N2 as the carrier gas. The temperatures of the oven and ECD were 150°C and 250°C, respectively. Gas fluxes were calculated assuming a linear increase or decrease of gas concentrations in the chambers. The flux rate was calculated using the equation:

$$F = kd\left(\frac{273}{T}\right)\left(\frac{V}{A}\right)\left(\frac{\Delta C}{\Delta t}\right)$$

Wherein where F is the rate of gas emission ($mass/ha/d$), k is derived from a linear relationship between gas fluxes and temperature (1.44×10^6 for $CO_2 - C$), d is the gas density ($^g/_{cm^3}$) at 273 K and 0.101 MPa ($5.36 \times 10^{-4} {}^g/_{cm^3}$ for $CO_2 - C$), T is the air temperature (K) within the chamber, V is the volume of air within the chamber (cm^3), A is the area of the base of the chamber (cm^2), and $^{\triangle C}/_{\triangle t}$ is the is the average rate of change of gas concentration over each time interval.

Analysis of variance was performed using PROC MIXED repeated measures model

(SAS v. 9.3, SAS Institute, Cary, NC). Treatments within individual sampling events were compared using a Student's t-test. Treatment effects were considered significant when P value was less than 0.05.

3.2.3 Nitrogen Mineralization and Litter Decomposition

The impact of raw milk on soil nitrogen dynamics and organic matter decomposition was investigated by destructively sampling equivalent experimental units. The experiment was laid out in a completely randomized design with two experimental treatments, and five sampling period, each with six replicates. The treatments were: i) foliar application of raw milk at the rate of $18.7\,mL\,m^{-2}$ and (ii) a control treatment with no liquid application.

Two hundred grams of fresh soil (approx 176 g dry wt.) was loosely packed into square plastic pots, 7.6 cm wide $\times$6.3 cm tall. To measure rates of decomposition of organic material, a small quantity (approx. 0.25 g) of organic material (chopped grass litter finely ground) contained within a small cylinder of fiberglass screen material (1.6 by 1.8 mm mesh), was buried 1 cm beneath the surface of the microcosm selected to be destructively sampled after 14, 21, and 28 days. To allow the biochemical processes within the soil to stabilize, microcosms sat for three weeks before treatment was applied. Over the course of the experiment, equal amounts of water were added to the soils every 2-3 days to maintain the soil water contents close to field capacity (between 12 and 15%, dry wt. basis). Raw milk, diluted 50 times was sprayed onto the soil surface of half of the microcosms at concentrations equivalent to the recommend field application rate; the remaining half received deionized water.

Microcosms were destructively sampled 1, 7, 14, 21, and 28 days after milk application. During each sampling event, the "litter bags" were removed, carefully washed, dried at $60°C$, and weighed. The soil was thoroughly mixed and immediately dried

at $60°C$. Soil mineral N concentrations ($NH_4 - N$ and $NO_3 - N$) were determined using $1M$ KCl extracts using a Lachat QuikChem AE flow-injection auto-analyzer.

Treatments effects were tested using an overall and sampling day-specific ANOVA (JMP v. 9.30 SAS Institute, Cary, NC). Treatment effects were considered significant when P value was less than 0.05.

3.2.4 Forage Growth

The impact of raw milk on a variety of forage growth parameters was investigated. The experiment was laid out in a randomized block design with two experimental treatments, each with six replicates, blocked by two container sizes. The treatments were: i) foliar application of raw milk at the rate of $18.7\,mL\,m^{-2}$ and (ii) a control treatment with no liquid application.

Cylinders were constructed of clear high density polyethylene (HDPR) pipe wrapped with removable opaque black plastic. Cylinders were marked every 3 cm along their length; these line would be used to estimate root density at various depths. A cap with holes at the bottom of each cylinder ensured there was no soil loss but allowed for the free passage of soil leaches from the microcosms. Two different size containers were used; large microcosms were 7.8 cm (inside diameter) by 30 cm; small microcosms were 7.1 cm (inside diameter) by 25 cm (3.1). Large and small microcosms contained 2000 g (approx. 1760 g dry wt.) and 1375 (approx 1210 g dry wt.), respectively, of sieved, gently packed, soil.

Perennial ryegrass *(Lolium perenne* L, var 'Boost') were planted 0.5 cm below the soil surface in each cylinder. After emergence, plants were culled, so that each pot contained thirty healthy plants. The cylinders were arranged in a randomized design and placed in a University of Vermont temperature-controlled greenhouse chamber with a 16/8 h light/dark cycle and a mean temperature of 25°C (day) and 16°C

(night). Raw milk, diluted 50 times was sprayed onto the soil surface of half of the microcosms at concentrations equivalent to the recommend field application rate; the remaining half received deionized water. Once a week, microcosms were brought to field capacity to simulate natural drying and wetting cycles.

Root growth was monitored by counting the number of times roots intersected the lines along the outside of the cylinder, 7, 14, 21, and 28, after treatment application. Shoot length was measured on five randomly selected tillers within each cylinder 18, 24, 29, 37 and 44 days after treatment application. Values would be used to calculate shoot elongation rate over time.

Grasses were first harvested 20 days at a height of 6 cm above soil surface. Each sample was vigorously rolled between researcher's hands for 15 seconds to form a tight ball; the sap was then extracted used a garlic press. Brix values for each batch were measured immediately using a Vee Gee Scientific STX-3 Handheld Refractometer.

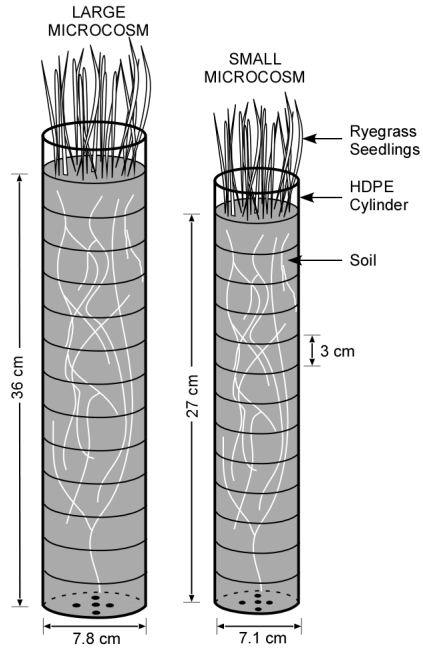

Figure 3.1: Diagram of the soil microcosms used in greenhouse study of forage growth.

Grasses were allowed to regrow for 25 days before being cut to surface level. During both harvest events, the number in tillers in each cylinder was recorded and the shoots were oven dried at $60°C$ to determine above ground biomass production. After the final harvest, the proportion of dead/diseased forage within each microcosm was measured using digital image analysis. Sampled were photographed in a lightbox. Once acquired, images were analyzed using ImageJ 1.453 (National Institutes of Health, USA). Percent dead dry matter estimates were determined by dividing the number of brown pixels by the total number of brown and green pixels. The roots were separated from the soil, over dried, and weighed to determine belowground biomass.

Analyses of variance was performed using PROC MIXED repeated measures model (SAS v. 9.3, SAS Institute, Cary, NC) for root intersection count and shoot elongation data. Treatments within individual sampling events were compared using a Student's t-test. Treatment effects were considered significant when P value was less than 0.05.

3.3 Results

3.3.1 Soil Respiration

In general, the rate of soil respiration decreased over the course of the study in the amended soil and remained fairly constant in the unamended soils. The amended soil had a significantly higher soil mean respiration rate ($P<0.001$) (Fig. 3.2). The soil respiration rate within microcosms treated with milk was not significantly different from controls on any one day of the study. The overall repeated measure ANOVA did not detect any significant influence of the treatment on carbon dioxide flux rate ($p<0.58$ for amended soil and $p< 0.47$ for unamended soil).

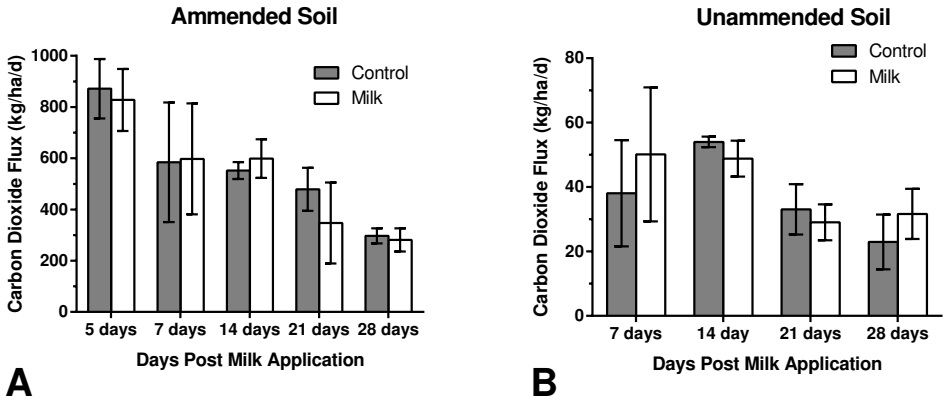

Figure 3.2: Mean carbon dioxide flux rate of A, amended with leaf litter, and B, unamended soils treated with raw milk. Error bars represent one standard deviation from the mean.

3.3.2 Nitrogen Mineralization and Litter Decomposition

Nitrate concentrations were low (<5 mg/kg) during the first sampling event one day after the treatments was applied in both the control and milk treatments. They remained consistently higher for the remaining duration of the study in all microcosms. The overall ANOVA did not detect any significant effects of the treatment on nitrate-N concentrations nor were the concentrations significantly different on any single day (Fig.3.3) ($F<0.65$).

Ammonium-N concentrations were fairly consistent over the duration of the study in the microcosm soils. One day after the treatments were applied, the plots receiving milk had significantly greater ammonium-N concentrations ($P<0.0133$) (Fig.3.3). The overall ANOVA did not detect any significant effects of the treatment on nitrate-N concentrations nor were the concentrations significantly different on any other single day ($F<0.2263$).

The litter buried in the surface of the soil microcosms lost 2.3% per day among controls and 1.8% per day among those treated with milk. The application of milk had no affect on the rate of decomposition ($F<0.9009$).

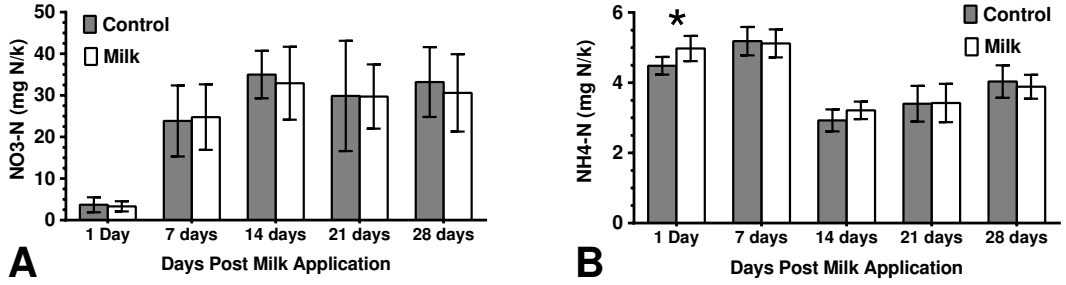

Figure 3.3: Mean soil nitrate-N (A) and ammonium-N (B) concentrations in soil microcosm (n=7). Asterisk indicates significant difference from control at P< 0.05.

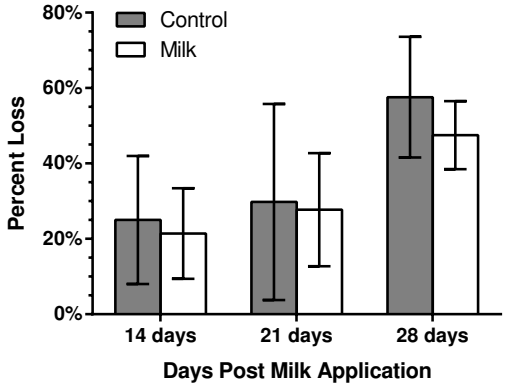

Figure 3.4: Decomposition of litter from litter bags in soils treated with and without raw milk displayed in terms of percent loss.

3.3.3 Forage Growth

The rate of shoot elongation declined steadily over the course of the experiment within both treatment groups (P<0.001). Elongation rates ranged from a mean of 0.56 to 0.12 cm/day. However, there was no significant milk effect (F=2.71; P<0.136). Plants within the cylinders treated with milk tillered significantly more rapidly than plants in the control immediately following the milk application (p<0.0184) (Figure 3.5-A). However, the rate of tillering between days 21 and 46 after milk application were not significantly different between the two treatments. Mean tiller mass (above ground biomass divided by the number of tillers) did not differ between treatments

during either of the harvest events. Means, standard deviations, and P-values for each paramter are displayed in Table 3.2.

Cylinders amended with milk produced significantly greater aboveground biomass at the first sampling event, 21 days after milk application (Figure 3.5-B). The brix value of the forage was similar within treatments. Following the second harvest, 26 days later, there was no treatment effect. In all cylinders, substantial portion of forage browned approximately 30 days after treatment application, owing partially to fungal diseases and partially to a soil nitrogen deficiency. There was no difference between the proportions of dead or brown forage within cylinders.

Over the course of the experiment, root density was estimated by counting rooting intersections and specific depths. After treatment application total number of intersections at all depths increased steadily at the rate of approximately 18 intersections per day. After 30 days, root inspection counts were terminated because of condensation within the walls of chamber inhibited counting. The treatment had no effect on the rate of at which roots grew within cylinders. After 30 days, more root intersections were counted in the cylinders treated with milk than the controls (Figure 3.6). There was no treatment effect on any other day of the study. Shoot: Root ratio within cylinder was approximately 1:2 and was not influenced by the treatments. The mass of the roots was also not influenced by treatments.

Table 3.2: Measures of ryegrass plant growth in soil microcosms treated with milk. Mean ± SD (n=6). Asterisks indicate significant differences between the chemical treatments and controls for each sampling event.

Parameter	Control	Milk	P-Value
Shoot Elongation Rate (cm/day)	0.42 ± 0.12	0.34 ± 0.05	0.1306[1]
Tillering Rate			
First Harvest (tillers/day)	$0.28 \pm .23$	0.68 ± 0.26	**0.0184***[2]
Second Harvest (tillers/day)	0.27 ± 0.16	0.25 ± 0.08	0.7970[2]
Mean Tiller Mass			
First Harvest (g)	0.009 ± 0.002	0.009 ± 0.001	0.9244[2]
Second Harvest (g)	0.022 ± 0.002	0.021 ± 0.002	0.5925[2]
Above ground biomass			
First Harvest (g)	0.31 ± 0.03	0.39 ± 0.04	**0.0073***[2]
Second Harvest (g)	0.91 ± 0.13	1.02 ± 0.16	0.1890[2]
Dead Above Ground Biomass (%)	55 ± 4	54 ± 4	0.8141[2]
Forage Brix Value (%)	13.82 ± 0.75	13.75 ± 0.42	0.8174[2]
Below Ground Biomass (g)	1.84 ± 0.26	2.09 ± 0.36	0.1983[2]
Root / Shoot Ratio	0.50 ± 0.03	0.50 ± 0.07	0.9993[2]
Root Growth Rate (Intersection/day)	17.59 ± 1.66	19.09 ± 1.74	0.1566[1]

[1] Calculated using Proc Mixed Repeated Measures ANOVA comparing treatment and control over multiple days

[2] Calculated using student's t-test comparing treatment and control for each sampling event

3.4 Discussion

It has been speculated that raw milk may be "potent microbial fertilizer" with forage growth promoting properties (Gompert and Richardson, 2011). Pre-treating seeds with diluted raw milk, amino acids in milk, and bacteria in milk can significantly enhance plant growth and productivity (Sudisha et al., 2011; Nautiyal et al., 2005). The authors hypothesized that bacteria and amino acids in the milk positively influenced soil biogeochemical properties. There is a dearth of scientific evidence regarding the effectiveness of raw milk as a forage biostimulant and the mechanisms at work. This chapter is the first to examine the effect of milk on select biochemical soil processes and to evaluate the effect of raw milk and specific plant growth variables.

The milk treatment had no effect on litter decomposition rate as measured using

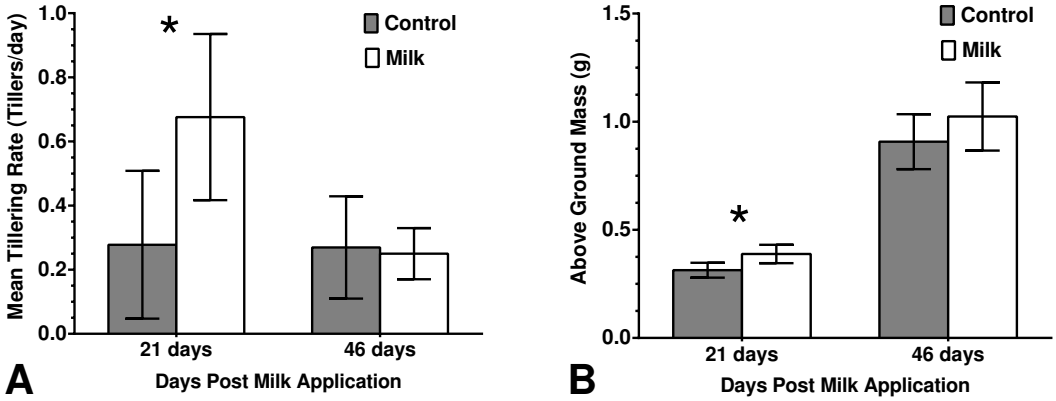

Figure 3.5: (A) Mean tillering rate (tiller per day) of perenial ryegrass between 0 to 20 days and 21 to 46 days after treatment application. (B) Mean above ground mass (g) of ryegrass within pots 20 days and 46 days post milk application. Error bars represent one standard deviation from the mean. Grasses were cut to 6cm 20 days and allowed to regrown. During the second cut, grass was cut to ground surface. Error bars represent one standard deviation from the mean. Asterisks indicate significant differences from controls at $P < 0.05$.

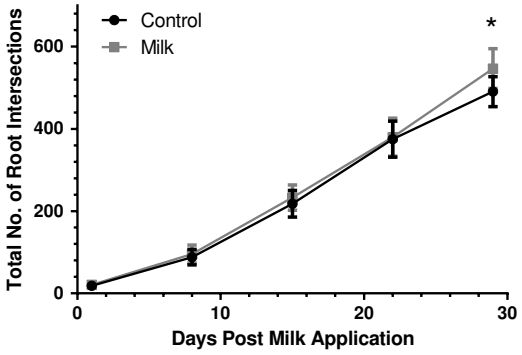

Figure 3.6: Mean total number of root intersections in each cylinder. Error bars represent one standard deviation from the mean. Asterisks indicate significant differences from controls at $P < 0.05$.

the buried litter bags. These results are not consistent with other scientific findings. Chen et al. (2003) applied an amino acid biostimulant containing proprietary fermentation products and trace minerals to soil at the rate of $0.005\,\mu l\,g^{-1}$ soil[1] and measured the impact on litter decomposition using a very similar method; the treatment increased the decomposition rate of wheat straw more than twofold compared to the control. The application of milk, at the recommended rate, would add approximately $0.02\,\mu l\,g^{-1}$ of amino acids – appreciably more than the rate applied by Chen et al. (2003). Similarly, Higa and Kinjo (2000) showed that minute addition of lactic acid fermentation bacteria enhanced the rate of decomposition of woodchips in soil. The failure of this study to detect a difference between the treatments may be due in part to experimental error; the litter was very finely ground, and some material was lost whenever the litter bags were moved. As a result, the data was highly variable.

There was no measured significant treatment effect on soil basal respiration rates from the amended or unamended soil. No studies have measured the impact of biostimulants on basal respiration rates. Sonnleitner et al. (2003) applied whey to soil microcosms at a concentration equivalent to 1% of soil dry mass and reported discovered a 4.6 fold increase in basal respiration rate. This represents an application rate 350 times greater than the one employed in our study. The lack of significant results discovered in this experiment could be a consequence of under-sampling; either the treatment effect was only evident immediately after the milk was applied or there were not enough replicates to detect a difference. It is also possible that the application of milk at the rate of $18.6\ g\,m^{-2}$ was not great enough to induce a change. In either case, the results indicate that milk treatment does not affect microbial activity and decomposition rate in soils rich and poor in carbon and nitrogen.

[1]Based on the assumption that the chemicals would be incorporated into the surface 2 cm of soil, with a bulk density of approximately$1.2\,g\,cm^{-3}$

Soil nitrate-N concentrations were not influenced by the treatment. The milk caused ammonium-N concentrations to spike 0.5 $mg\,N\,kg^{-1}$ the day after the treatment was applied. Given that nitrogen was applied at the rate of approximately 0.003 mg/kg[2], the increase can be attributed to a change in the soil biogeochemical processes. Chen et al. (2002) found that amino acid biostimulants significantly decreased soil ammonium concentration and increased soil nitrate concentration 7, 14 and 28 days after the treatment application. The difference between our results, and those of Chen et al. (2002) alludes to the possibility that the microbes in milk influenced nitrogen mineralization processes. The treatment had no effect on soil nitrogen concentration 7, 14, and 28 days after the treatment application, therefore any positive effect initiated by the milk would be very brief.

The milk treatment significantly increased grass tillering rate and aboveground biomass immediately after application. Average tiller mass was not affected by the treatment, therefore the increase in above ground biomass was the consequence of increased tillering rate and not increased tiller height or thickness. The mechanism responsible for promoting tillering are complex, multifactorial, and not fully understood (Assuero and Tognetti, 2010). There are several possible explanations for the observed increase in number of tillers. Willmoes et al. (1988) observed that additions of sucrose increase tillering rate; the sugar in milk may have a similar effect. Niranjan et al. (2004) observed the inoculation of soil with *Pseudomanas spp.*, a bacteria found in high concentration in milk, increases tillering rate in pearl millet. Additional studies would need to be conducted to determine the exact mechanism.

The preliminary studies conducted as part of this experiment allude to the potential of milk to be potent biostimulant. Milk appears to increase grass tillering rate.

[2]Assuming a milk protein content of 3.22% (None, 2012) and a nitrogen to protein conversion factor of 6.38 (Tontisirin, 2002)

However, before any conclusion can be drawn, additional studies with more replicates and sampling events are prudent.

CHAPTER 4

OVERALL CONCLUSIONS

Summary of key findings In the field experiment, the application of raw milk onto pastures significantly influenced certain forage parameters; however, there was no consistant trend across sampling events or farms. The gains recorded are neither great enough to influence milk production nor consistent enough to be a reliable solution for pasture degradation. Most likely, the significant results observed represent statistical anolomies. In the greenhouse trials, grasses treated with raw milk tillered significantly more rapidly than grasses which did not receive the treatment, significantly increasing above ground forage biomass. Other measured forage growth parameters including below ground biomass, leaf elongation rate, and forage brix content were not impacted by the treatment. In other labrotory based experiments, raw milk had very little impact on nitrogen mineralization and no impact on soil basal respiration rate or litter decomposition rate.

Implications Farmers applying milk to their pasture may want revaluate their reasons for doing so. For farmers with large quantities of waste milk, land application is the most environmental friendly means of disposing of the milk. However, farmers should not expect the milk application to positively impact their forage production or quality. The meager gains recorded as part of this experiment are neither great enough to influence milk production nor consistent enough to be a reliable pasture

ammendment. Spraying the milk on pasture remains a viable means to dispose of milk but should not be relied upon as a pasture biostimulant.

We recommend that those wishing to experiment with raw milk on their own farm should spray the solution immediately before a rainstorm and after the forage was grazed to maximize the amount of milk reaching the soil. As milk also appears to stimulate grass tillers, farmers experimenting with this practice may want to consider spraying milk on recently seeded paddocks or on pasture with low plant density. Although farmers probably should not expect a substantial change in forage production and quality, it may positively affect pastures in ways not measured during this experiment.

Strengths and limitations The field study sampling regime was thorough enough to conclude, with a high degree of certainity, that raw milk had no significant impact on forage mass, ADF, NDF, and forage protein content. As these are the parameters which most influence milk production, these are important findings. In addition, through the greenhouse trials we were able to determine that raw milk stimulates grass tillering and nitrogen mineralization when applied to soil. These studies ellicited the possible mechanism of milk that warrents future considerations.

Despite the power of the field experiment, it was limited in its scope. Only two farms, both with good forage and excellent soil quality, were examined during a single field season. It is possible that a stimulatory effect would have been observed after a longer period or on a lower quality soil. The dry conditions present during the summer of 2012 may have inhibited any stimulatory effect which milk might otherwise have incurred. It is unlikely that the milk sprayed onto plant leaves was washed into the soil via a natural precipitation event. In addition, under the droughty summer conditions soil microbial activity and nutrient cycling would process slowly. The dry conditions

may have inhibited the movement of the beneficial bacteria into the soil thereby negating the potential for milk to positively influence soil and forage parameters. Furthermore, the unusually dry conditions may have inhibited any response resulting from microbial stimulation.

Through the laboratory experiments, we were able to ascertain a mechanism of action. However, most of the trials had a low power to detect significant differences. In addition, microcosms were infrequently sampled and treatment effects may have been missed.

Future research To make any definative conclusions regarding the merits of raw milk as a pasture ammendement, additional field and labrotory trials need to be conducted. Field trails need to span multiple years and include a greater number of farms with more diverse conditions. Specifically, raw milk trials need to be conduted under weather conditions that ensure the milk comes in contact with the soil and on farms with lower quality forage and soil. We hypothesize that the raw milk would be more likely to illicite a response under these conditions.

We would recommend repeating the greenhouse experiments with more replications and with greater variety of plant species and soil qualities. To definatley determine if the milk is acting on soil microbes, an experiment should be conducted on sterile soil. The forage growth, soil respiration, and nitorgen mineralization studies should be repeated with more frequent sampling especially within the first week post milk application.

Appendices

APPENDIX A

EXPLANATION OF FORAGE QUALITY

Forage quality can be defined as the extent to which a forage has the potential to produce a desired animal response (Ball, nd.). For a dairy operation, the most accurate measure of forage quality is the amount of milk produced per cow, per day. However, it is often not practical to make management desicisons nor complete scientific studies on the basis of milk production. As a result, many forage quality studies focus on factors which affect forage quality.

In the broadest sense, forage quality is a function of forage nutritive value and animal intake. The nutritive value refers to the concentration of available energy (total digestible nutrients), crude protein, minerals, anti-quality factors, and forage digestibility (the extent to which forage is absrobed as it passes through the animal's digestive tract). Nutrative value is influenced by a wide variety of factors including forage species, growth conditions, soil quality, and forage maturity stage. A wide range of data regarding forage nutritive value can be obtained though a forage nutrient laboratory analaysis.

Forage quality is also contingent upon animal intake. Because cows selectively graze fields, it is important to consider the palatability of the forage. For example, many weeds may have a high protein and nutrient content, however, because cows tend to avoid eating weeds, their presense in pastures lowers the forage quality. Pal-

itability is harder to assess but is influenced by plant species, moisture content, pest infestation, and compounds in the forage resulting in a sweet, sour, or salty taste. Frequently forage nutritive value and palatability are related.

Forage quality is the most important determinant of milk production. In this study, forage quality was measured in the following ways:

- **Forage nutritive value** was assesed using infrared reflectance spectroscopy (NIR) by Dairy One Laboratory, (Ithaca, NY). Through this analysis we obtained information regarding forage protein, acid detergent fiber, neutral detergent fiber, and mineral content.

- **Ratio of grass to legumes to weeds** was assesed using hand seperations. The ratio of grass to legumes strongly influences nutritive value and palibility; forage rich in legumes generally contains less fiber, more protein and has a more favorable flavor, each of which contribute to higher quality forage quality (Newman, et al. 2009). Conversely, weeds tend to reduce quality in both palitability and intake.

- **Brix content** was measured to assess the forage solute (sucrose, fructans, minerals, proteins, lipids, pectins and acids) concentration. Farmers often use brix content as an estimate of the forage sugar content. Grasses high in sugar increase the efficiency of milk production in animals (Moorby, 2001).

- **Percent standing dead matter** was used to assess the impact of heat and/or water stress and disease pressure on the forage. Compared to green, living, or productive forage, brown forage has significantly less nutritive value. During leaf death, soluble energy and protein contained in the old leaves are translocated to the roots, new leaves, or stem. Studies have demonstrated that the remaining

brown leaf is usually less than 45% digestible with lower crude protein and nutrient content (Calvert and Engel, 1982; Beaty and Engel, 1980). While nutrient concentrations vary as little as 10% over a season within green or brown fractions, they often differ by 50% between fractions (Beaty et al., 1979). Beaty et al. (1978) concluded that the amount of dead forage in a sample had more impact on the digestibility to tall fescue than varying nitrogen application rates, clipping heights, and clipping schedules. Consequently, variations between the green:brown ratios can be used to accurately predict animal gains (Beaty and Engel, 1980).

APPENDIX B

EXPLANATION OF TREATMENT ASSIGNMENT

In the field trials, treatment assignment within the paddocks was non-random. At site 1, systematic random treatment assignment was required in order to ensure that both treatments would be grazed simultansously. By generating wide treatment swaths, the farmer had some flexibily to generate paddocks of different sizes.

At Site 2, treatments were also assigned in a systematic random fashion; treatment was randomly assigned in the first paddock and alternated in subsequent paddocks. These created some uniformity in treatment assignment methodology between sites. In addition, alternating treatments also reduced the potential for the existing field scale forage trends to influence the results. Using farmer statements and visual estimates of forage mass, we speculated that forage pregrazing mass was signifiacntly lower on the south end of the pasture compared to the north end of the pasture but did not differ betewen the east and west sides of the pasture. Results from the first sampling event confirm these observations (Figure B.2A and B.2B). As a result, it seemed appropiate to alternate treatments throughout the pasture.

Alternating treatments had one unexpected consequence; because the farmer grazed even numbered paddocks during the night and odd numbered paddocks during the day, each group always grazed paddocks with the same treatment confirguration. In addition, water tanks were only located on the east side the paddock. Cows graz-

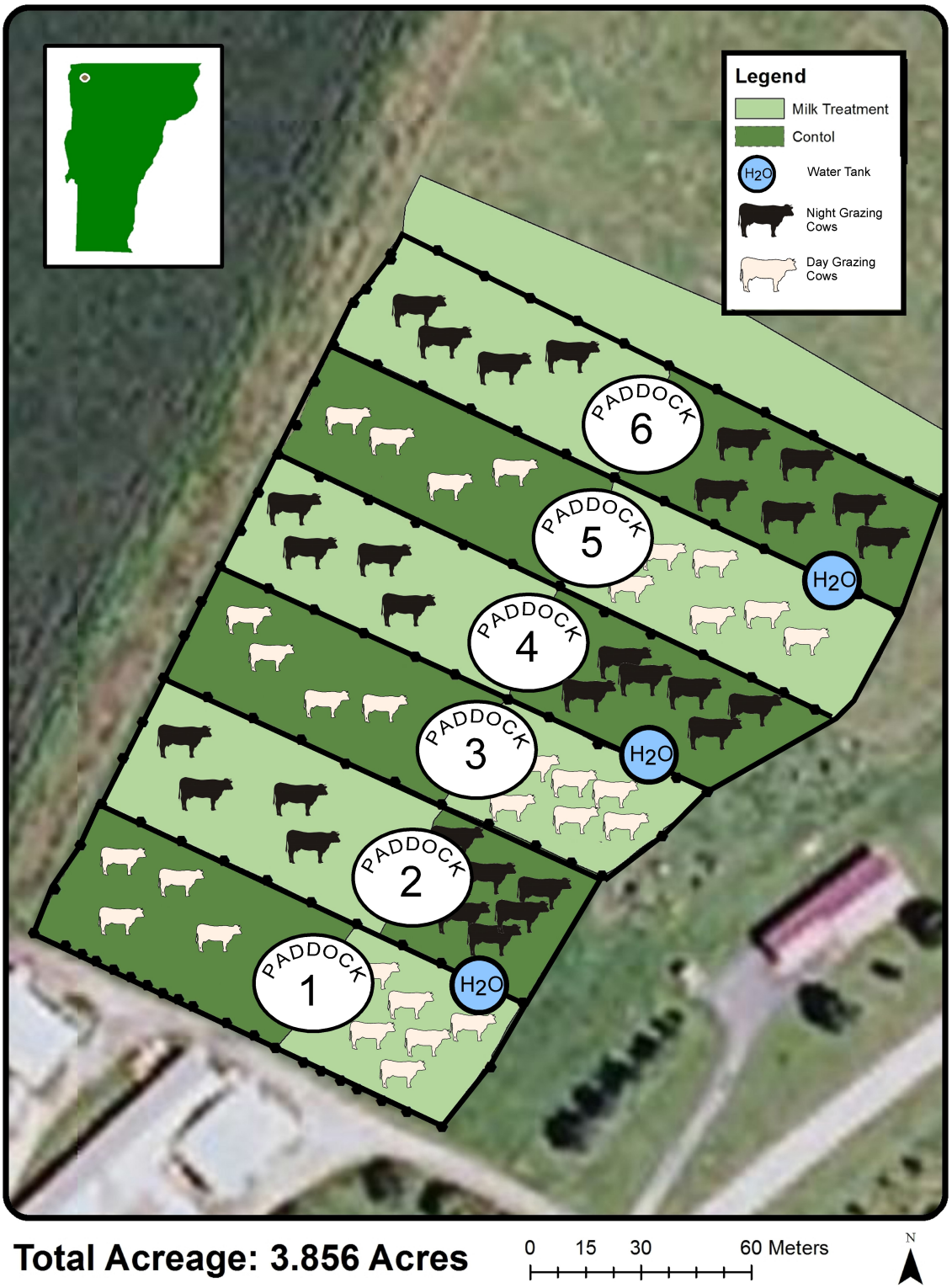

Figure B.1: Layout and grazing dynamics at Site 2. Treatments were paired within each paddock. Paddocks were grazed by two seperate groups of cows during either the daylight or night hours.

ing at night may have different water consumption habits compared to cows grazing during the day; one would expect cows grazing during the day would preferentially graze closer to the water as result of the heat. Figure B.1 illustrates this arrangement.

We were able to determine if the placement of the water tanks or the groups of cows influenced forage consumption. The forage consumption within experimental units near the water tanks was compared against experimental units on the far away from the water tanks (Figure B.3A); there was no significant difference (p=0.6001). Forage consumption of cows grazing at night was also compared against cows grazing during the day (Figure B.3B); again there was no significant difference (p=0.400). Therefore, we can conclude that the decision to alternate treatments most liklely had no effect on the results.

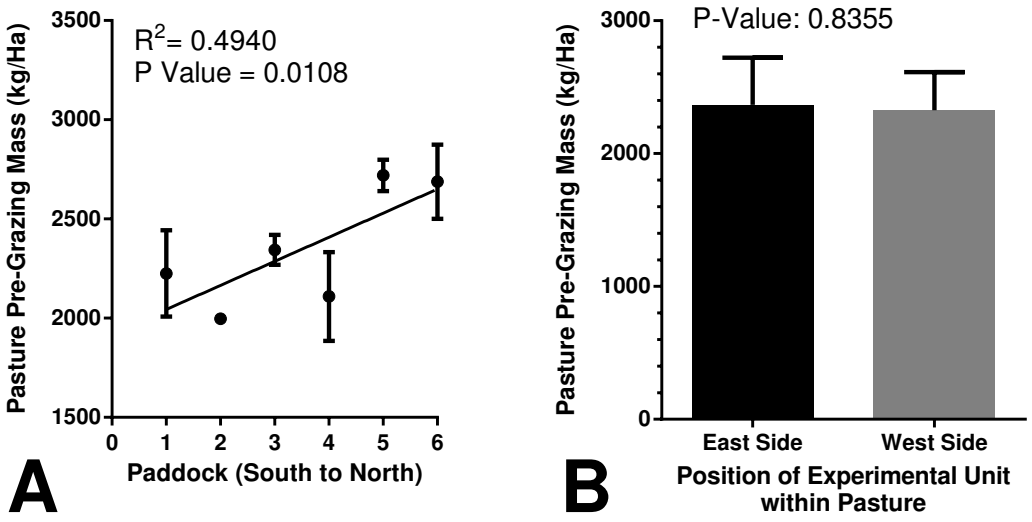

Figure B.2: Relationship between experimental unit position within pasture and pasture pre-grazing mass at Site 1 during the first sampling event. Figure A displays the pre-grazing mass in each paddock from south to north; data analyzed using simple lineral regression. Figure B displays the pre-grazing mass in the experimental units on the east and west side of the paddock (n=6); data analyzed using a student's t-test.

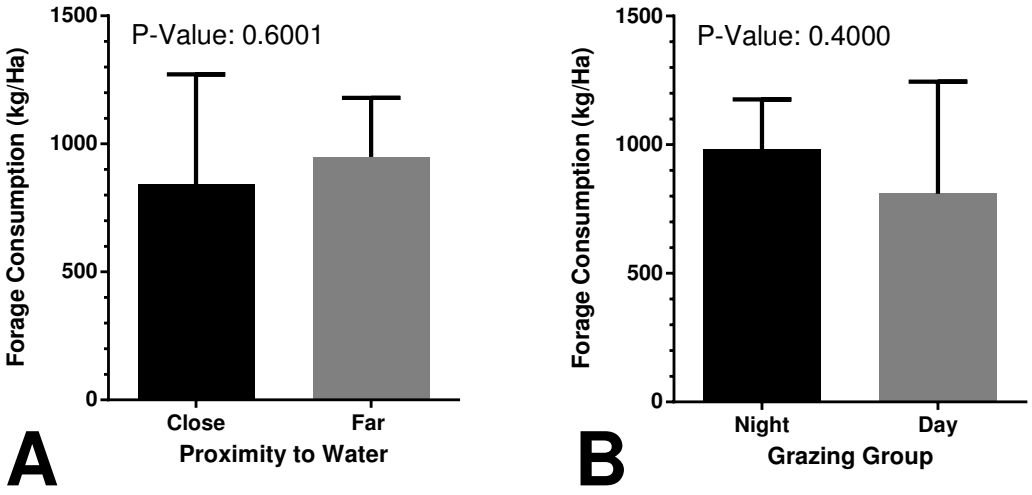

Figure B.3: Relationship between experimental unit position within pasture and forage consumption at Site 1 during the first sampling event. Figure A displays the mean forage consumption in experimental units close to and far from water tanks; data analyzed using a student's t-test. Figure B displays the mean forage consumption in paddocks grazsed during the night and during the day (n=3); data analyzed using a student's t-test.

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