AN ABSTRACT OF THE THESIS OF

Lorena Fernanda Carmona Flores for the degree of Master of Science in Animal Science presented on January 21, 2020.

Title: Effects of Diversity and Spatial Separation of Pastures on Forage Production, Milk Yields, N Partitioning and Methane Emissions

Abstract approved: ____

Serkan Ates

Diverse pastures containing multiple species help extend the grazing season and reduce the reliance on one or two species to meet all the nutritional requirements of livestock. Planting pasture species in spatially separated adjacent strips can increase the proportion of pasture plants with certain agronomic and nutritional attributes such as tolerance to waterlogging and presence of condensed tannins. The higher pasture quality of pasture plants with high bioactive compounds often lead to improved milk yield and reduced environmental impact of dairy farming. Thus, in the current study, combinations of simple and diverse pasture mixtures in mixed and spatially separated pasture strips were evaluated for their effects on forage DM production (Chapter 3), DM intake, milk yields, N partitioning and methane emissions (Chapter 4). The study was conducted between 2017 and 2019 in Corvallis, Oregon. A 7.2-ha paddock was divided into three 2.4-ha blocks to serve as replicates for the experiment. Each block was divided into 4 subplots of 0.6 ha, which were randomly allocated to the following treatments: 1) a simple pasture mix (perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)); 2) a simple pasture spatially separated; 3) a diverse pasture mix (perennial ryegrass, festulolium (X *Festulolium braunii*), white clover, birdsfoot trefoil (*Lotus corniculatus*), plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*)) or a 4) diverse pasture spatially separated. Simple pasture mixes were composed of perennial ryegrass and white clover, while diverse pasture mixtures contained perennial ryegrass, festulolium, white clover, birdsfoot trefoil, plantain and chicory. Total annual DM production of pastures in 2017-2018 growing season ranged from 9.0 to 11.5 t DM/ha. The effect of diversity on biomass production was not significant ($P=0.17$) while mixed pastures appeared to have greater DM yield than spatially separated pasture mixtures by 2.97 t DM/ha/y ($P=0.07$). The grazing trial was conducted for 21 days from 3 April to 24 April in 2019. Thirty-six mid-lactation Jersey cows were randomly assigned to one of four pasture treatments. Cows that grazed diverse pastures had higher ($P<0.05$) milk solids (2310 g/d) and milk protein ($P<0.01$; 883 g/d) yields as compared to those that grazed simple pastures (2083 g/d and 778 g/d, respectively). Spatial separation did not affect ($P>0.05$) DMI, milk yield, or milk components except lactose content of milk, which was lower ($P<0.01$) in spatially separated pastures. Although diversity did not affect ($P=0.22$) daily methane production (g/d), cows that grazed diverse pastures had lower ($P<0.05$) methane yields per DM eaten as compared to simple pastures. Cows that grazed diverse pastures had lower urine N (%) and urea content and lower daily N output through urine. Thus, pasture species diversity can increase the pasture yield in periods when perennial ryegrass and white clover are less productive and had positive effects on milk solid production and environmental impact due to decreased urine N output and methane emissions.

Keywords: species diversity, DM intake, methane emissions, N partitioning, plant secondary compounds

Effects of Diversity and Spatial Separation of Pastures on Forage Production, Milk Yields, N Partitioning and Methane Emissions

by
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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented January 21, 2020
Commencement June 2020

Master of Science thesis of Lorena Fernanda Carmona Flores presented on January 21, 2020

APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lorena Fernanda Carmona Flores, Author

Acknowledgements

Thank you to the Lord because He showed me that I can do all things through Christ who strengthens me.

I would like to thank the Agricultural Research Fund for funding this research and the Oregon State University Department of Animal and Rangeland Sciences for funding my graduate program.

I also would like to express my gratitude to my advisor, Dr. Serkan Ates for all the emotional, physical, intellectual and financial support during my study. I would like to acknowledge my committee members, Drs. Massimo Bionaz and David Hannaway for their guidance and support in planning and carrying out my research. And thank you to Dr. Sol Morales (Chile) for helping me to get the opportunity to study in the USA.

Thank you to all the graduate student (Muhammet, Yunus, Hunter, Sebastiano, Angela, Mussat and Matthew) and undergraduate students (Diana, Clara, Carin, Julia, Elizabeth, Emily, Emilia, Madelein, Ruben, Danielle, Samantha and Kylie) who helped me in conducting the grazing experiment. In particular, I would like to recognize my fellow student, Randi Wilson, for her friendship and solidarity that made the work enjoyable and helped me get through the hard times. Thank you to the people from the OSU dairy farm and to the veterinarians Jorge Vanegas and Joe Klopfenstein for monitoring the cow's health and helping with the sampling process.

Thank you to all my collage friends, Christian Graduate Fellowship Group, Corvallis Gospel Hall and Iglesia Biblica de Eugene for the good moments, which reminded me that there are more things to meditate about and live for besides my research.

Finally, I would like to thank my parents, Victor and Ester for all what they have granted me. This USA-adventure would not have been possible without their support.

"*He causeth the grass to grow for the cattle, and herb for the service of man.*" Psalm 104:14

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List of Abbreviations

ADF	Acid detergent fiber
BCS	Body condition score
BCT	Bound condensed tannins
BW	Body weight
CF	Crude fiber
CT	Condensed tannin
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FCM	Fat-corrected milk
GHG	Greenhouse gases
H_2	Metabolic hydrogen
MUN	Milk urea nitrogen
ME	Metabolizable energy
N	Nitrogen
N_2O	Nitrogen oxide
NDF	Neutral detergent fiber
OM	Organic Matter
PD	Purine derivatives
PM	Rising plate meter
PNW	Pacific Northwest
SCC	Somatic cell counts
SF6	Sulfur hexafluoride
SNF	Solid non-fat
TP	Total phenolic
UCT	Unbound condensed tannins
TMR	Total mixed ration

Chapter 1: Introduction

1.1 Introduction

Historically, grass-based cattle production was the main management practice around the world, but widespread availability of affordable concentrate-feed enabled dairy farmers to adopt more intensive-confinement production systems in order to increase and intensify milk production per animal. However, in the last decades, pasture-based livestock production in the US has been growing rapidly due to the increasing input costs of confined-systems and due to consumer preferences. Milk yield per cow from pasture-based-systems is lower than under confinement systems because the reduced intake of nutrients in grazing systems, since pastures provide 19% less dry matter (DM), organic matter (OM), and net energy compared to confinement TMR diets (Kolver & Muller, 1998). However, pasture-based systems can have lower operational costs and higher net income per cow (White *et al*., 2002) if high pasture use efficiency is achieved.

Increased nutritional value of pastures through increasing the content of legumes- by planting pure swards of grass and clover within the same field (spatial separation) has been reported by Marotti (2004), Chapman *et al.,* (2007), and Solomon *et al.,* (2011). This higher nutritive value in the pasture may increase the intake of the grazing cows, and in consequence improve milk production. Also, the inclusion of diverse plant species in the pasture might increase the DM yield of the pasture and improve the milk yield (Nobilly *et al*., 2013).

In addition to the need to be more efficient in production, dairy farmers are also concerned with minimizing their environmental impact, recognizing that agriculture contributes about 11% of all global greenhouse (GHG) emissions (Rotz 2018), and nitrogen (N) losses from dairy systems can be an issue (Grainger *et al.,* 2009). Secondary metabolites, like condensed tannins, present in forage species such as plantain, chicory, and birdsfoot trefoil (Totty *et al.,* 2013) (Li & Kemp, 2005) can benefit the environment through a change in the excretion of N from the urine to feces in dairy cows (Ghelichkhan *et al*,. 2018) and reduce enteric methane production (Woodward *et al.*, 2004).

Although there have been some research reports on the use of spatially-separated plantings to increase the content of legumes in the pasture (Marotti 2004; Chapman *et al*., 2007), these studies have been done using only two species, and they measured only milk production as an indicator of treatment success. The present study evaluated the production and environmental effects of spatially-separated plantings with multiple forage species.

1.2 Research objectives

The purpose of this study was to develop sustainable high performing pasture-based dairy production systems where desirable pasture traits for animal performance are maintained at a high abundance in the diet. Specific objectives of the study were to:

1. Assess the effects of pasture species combinations on environment indicators, milk production, and grazing behavior of dairy cows.
2. Determine annual and seasonal production, nutritive value, and botanical composition of forage in simple and diverse pastures in mixed and spatially separated plantings.
3. Quantify the N concentration of milk, blood, feces, and urine of the cows to detect differences in urine on each pasture type.

1.3 Hypotheses

Hypothesis 1: Increased pasture species diversity through including compatible forage species in mixtures will improve the annual DM yield and extend the growing season.

Hypothesis 2: Increased proportions of plants with high bioactive compounds on pasture on offer will increase and milk yield of cows and decrease N and methane emissions.

Hypothesis 3: Spatial separation of pasture species will increase dry matter intake and milk yield of cows.

Chapter 2: Literature Review

2.1 Forage Production of Dairy Pastures in the PNW

2.1.1 Prevailing agroecological conditions

The potential production and herbage composition of pastures are primarily governed by climate, soil characteristics, fertility, and topography. Agroecological conditions of the Pacific Northwest (PNW) are highly conducive to growing high quantities of high-quality forages. The temperate climate and winter-dominated precipitation conditions promote rapid vegetation growth during the cool season (late winter and early spring). The climate in the PNW is influenced by the Pacific Ocean, with a Mediterranean-type precipitation pattern of wet and temperate winter weather and a summer drought conditions. In this humid zone, the annual precipitation ranges from 450 mm to < 2000 mm (Shewmaker *et al.*, 2015). Plant growth commences in early spring with temperatures above 5 C and slows with hot, dry summer conditions. The autumn is typically short with warm temperatures and increasing rain showers (Fransen *et al*., 2017).

The most prevalent soil texture in western Oregon is silty clay loam. This soil type is characterized by low organic matter and is therefore susceptible to slaking (structural breakdown). Silty clay loam soils have a very thin surface crust that significantly reduces water entry into the soil; runoff and erosion are important problems in these soils (Huddleston & Kling, 2007).

One of the most common yield-limiting factors in crop growth in western Oregon is soil acidity. Acid soil pH is natural in western Oregon and certain farmers' practices (such as N fertilization) increase this acidification (Han *et al*., 2015). One of the ways that soil acidity limits pasture growth is through the reduction of symbiotic N_2 fixation of legumes. Low pH limits formation and function of nodules of this symbiotic process involving legumes and *Rhizobium* bacteria through increased plant-available Mn, which inhibit shoot growth, decreased phosphorus and molybdenum solubility, and reduced availability of Ca, Mg and K (Hart *et al*., 2013).

2.1.2 Key PNW pasture species

A diverse group of temperate forage species is grown in permanent and temporary pastures of western Oregon. Tall fescue (*Festuca arundinacea*), orchardgrass (*Dactylis glomerata*), perennial ryegrass (*Lolium perenne*), annual ryegrass (*Lolium multiflorum*), and small grain winter annuals are extensively cultivated for forage and seed production. Annual and perennial legumes such as white clover (*Trifolium repens*), red clover (*Trifolium pratense*), and birdsfoot trefoil (*Lotus corniculatus*) are often grown for grazing and conserved forage systems in combination with grass species and occasionally as monoculture stands (Shewmaker *et al.,* 2015). Alfalfa (*Medicago sativa*) production, common east of the Cascade mountains, is limited in the PNW due to high soil acidity and poor soil drainage. Chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) are often included in pasture mixtures due to their rapid spring growth rates, superior feeding qualities, and high bioactive compounds. Annual legumes species such as hairy vetch (*Vicia villosa*) and crimson clover (*Trifolium incarnatum*) are mostly utilized in cover crop mixtures to provide off-season grazing and to improve soil health.

The combination of perennial ryegrass and white clover is the most common dairy pasture worldwide. In temperate agro-ecologies, perennial ryegrass can be highly persistent and productive with high regrowth potential under frequent cutting and grazing management (Ostrem *et al.*, 2013). Similarly, white clover with its stoloniferous and prostrate growth is highly tolerant of intensive grazing management and forms a highly compatible mixture with perennial ryegrass (Eriksen *et al.,* 2012). However, the availability and nutritional quality of this pasture mixture can vary widely throughout the year, due to seasonal climatic changes. The combination of perennial ryegrass and white clover can yield between 7 and 14 t DM/ha forage per year depending on the edaphic and climatic conditions as well as the proportion of white clover in the pastures (Elgersma & Schlepers, 1997) . In the Mediterranean climate of Oregon, a simple pasture mixture (perennial ryegrass and white clover), with good grazing management, fertility, and soil moisture, has been reported to yield 22,000 kg DM/ha per year (Downing, 2018).

Although simple grass-clover pastures are commonly used due to the simplicity of management, several studies have reported the benefit of using diverse pasture mixtures (Woodward *et al*., 2013; Totty *et al*., 2013; Soder *et al*., 2006). Pastures containing multiple species help extend the grazing season and reduce the reliance on one or two species to meet all nutritional requirements of livestock. Additionally, certain agronomic and nutritional attributes of pasture plants, such as tolerance of waterlogging and presence of condensed tannins can be better provided in diverse pastures. Therefore, plant species such as birdsfoot trefoil, plantain, and chicory are increasingly included in pasture mixtures. These species can positively affect the pasture by increasing the forage production around the year, and, compared with perennial ryegrass, chicory and plantain have lower fiber and N and higher mineral content (Gregorini *et al*., 2013).

Birdsfoot trefoil is a non-bloating, short-lived perennial legume with high adaptability and yield potential (5.73-6.07 tons DM/acre) (MacAdam & Griggs, 2013) and on average has a CP content of 257 g kg^{1} DM (Li & Kemp, 2005). It is the most widely distributed Lotus species in the Mediterranean region and north Africa, and the second most widespread Lotus in the USA, Canada, South America, and parts Asia; this wide adaptability is due to tolerance of infertile acidic soils, tolerance of drought, and moderate winter hardiness (Ayres *et al*., 2008). The main problem of this legume is its difficulty to establish and poor competitiveness and persistence (MacAdam *et al*., 2006).

One special characteristic of birdsfoot trefoil is the presence of secondary compounds such as condensed tannins (CT) (36.1 g of CT/kg DM) (Li & Kemp, 2005). Condensed tannins are proanthocyanins that have the capability to attach to soluble proteins in the rumen and create insoluble CT-protein complexes. These protein complexes are not degradable in the rumen, therefore, they reach the intestine where they are digested with the rest of the non-degradable protein from the rumen. This process results in a decrease in the degradation of ruminal protein which decreases the formation of ammonia-N. This can benefit the environment through changing the excretion of N in dairy cows from urine to feces (Ghelichkhan *et al*., 2018). Another benefit attributed to CT in birdsfoot trefoil is the antiparasitic effect they can have on sheep which can increase body weight (Marley *et al*., 2003), positively affect wool growth, milk yields, and reproductive performance (Ghelichkhan *et al*., 2018), and reduce enteric methane production (Woodward *et al*., 2004). A dietary concentration of 0.17% CT/DM can positively affect the protein solubility in the rumen, but concentrations of 2-3% CT/DM are better for maximizing the nutritive benefits; values higher than 5.5% CT/DM inhibit microbial activity excessively and depress voluntary intake (Waghorn *et al*., 1990).

Plantain, a narrow-leaved, non-leguminous perennial herb (forb) is becoming an increasingly popular pasture species in both low-input sheep (dryland) and high input (irrigated) dairy pastures. The seedling emergence of plantain is quite rapid, but its low competitiveness can diminish its successful establishment when sown together with aggressive grasses (Stewart, 1996). It can yield up to 20 t DM/ha per year a under wide range of soil (pH 4.2 - 7.8) and climatic conditions (Stewart, 1996). An actively growing plantain can have 105-170 g kg^{-1} DM CP and 435 g kg^{-1} DM NDF concentration (Sanderson, *et al.* 2003). Plantain also contains high secondary metabolites and bioactive compounds such as talpol, acteoside, and aucubin (Navarrete, *et al.* 2016), some of them with diuretic properties that can represent great potential to reduce N leaching problems (O'Coneell, Judson, & Barrell, 2016). Condensed tannins can be present in small quantities in plantain (14 g of CT/kg of DM) (Totty *et al*., 2013).

Chicory is a perennial herb that has higher digestible organic matter in the summer and fall compared to grass-clover pasture mixes. Due to its seasonal growth (active in warm seasons, but dormant in winter), chicory can be used to improve pasture DM and nutrient intake in late spring, summer, and early fall (Muir *et al.*, 2014). One study (Li & Kemp, 2005) described that during favorable conditions, chicory can grow at 150 kg DM/ha/day. Chicory has more uniform and higher metabolizable energy (ME) (13.7 MJ/kg OM), CP (144-243 g/kg DM), and apparent OM digestibility (82%) compared to perennial ryegrass (12.3 MJ/kg OM; 201 g/kg DM; and 74%, respectively) (Soder *et al*., 2006). Although chicory also contains a small amount of CT (1.7-4.2 g of CT/kg DM, Totty *et al*., 2013), this is less than the minimum needed for preventing bloat (5 g of CT/kg DM) (Barry & McNabb, 1999).

Despite the benefits ascribed to chicory, a few studies reported decreases in DM intake of this herb (Foster *et al*., 2006). This was mainly attributed to the sesquiterpene lactone content of chicory, part of the defensive chemistry of the plant against insects. Sesquiterpene lactones can produce a bitter taste negatively affecting intake (Soder *et al*., 2006). Foster *et al*., (2006) reported that the acceptance of chicory by ruminant is inversely related to the total sesquiterpene lactone concentration. They also noted that the concentration of sesquiterpene lactone can vary depending on the cultivar, climate, and soil characteristics. In addition to the concentration of sesquiterpene lactone, other limitations of chicory are its winter dormancy, rapid reproductive stem growth during summer, and its low dry matter content (113 g/kg fresh weight), which can also restrict the voluntary DM intake (Li & Kemp, 2005).

The principal components of pasture and hay fields in United States are temperate perennial grasses (Burns & Bagley, 1996), including perennial ryegrass, orchardgrass, and tall fescue. Orchardgrass and perennial ryegrass are more palatable grasses for livestock compared to fescue, but tall fescue can be more persistent than orchardgrass in poorly drained acidic soils (<pH 5.5) (Filley, 2002). Tall fescue is the most commonly grown grass in the US because of its wide range of adaptation, ease of establishment, persistence under different management, tolerance of poor soil quality and various climatic conditions, and long grazing season with good winter growth (Schimidt & Osborn, 1993). However, there are several animal

performance problems associated with tall fescue, the most common being tall fescue toxicosis, which produce a decreased in the feed intake and milk yield, loss or low gain in weight, rough hair coat, and elevated respiration rate and rectal temperature (Strahan *et al.,* 1987). Furthermore, tall fescue is not a preferred grass species in most dairy systems due to its lower feeding value (nutritive value and intake) (Lee *et al*., 2018). Festulolium (x *Festulolium braunii*), a hybrid between ryegrasses and fescues was developed to improve the forage quality of tall fescue and meadow fescue. Festulolium has the potential of combining the higher forage quality of perennial ryegrass with the high persistency and stress tolerance of fescues (Ostrem *et al*., 2013). Festulolium can have higher dry matter yield than either tall fescue or annual ryegrass, and equal or higher crude protein (11.5%, 11. 5% and 9.8%, respectively) (Akgun *et al*., 2008).

2.1.3 Plant diversity and pasture composition

Achieving high forage utilization efficiency is of great importance for grazing-based dairy farms. However, farmers often plant simple pasture mixtures since they are easier to manage. Multispecies pastures are challenging with respect to seedling competition, herbicide applications and fertilizer requirements needed to maintain the desired botanical (Pembleton *et al*., 2016). The choice of a simple pasture combination leads to a little variety of plants in the pastures, with perennial ryegrass and white clover (grass-legume mix) being the two most used species around the world. Although this this combination can result in high pasture production (7-14 t/ha per year, varying according to the amount of white clover that the pasture contains) and N transfer from clover to ryegrass (Elgersma and Schlepers 1997; Roca-Fernández *et al*., 2016), neither of these species is tolerant to drought and 70% of the annual growth of perennial ryegrass occurs during spring (Rawnsley *et al.*,2007). This limits this mixture's climate suitability range and often results in seasonally low pasture quality (Nobilly *et al*., 2013).

In addition to the limited climate adaptation of the perennial ryegrass-white clover simple mixture, farmers also face other challenges, including the growing concern about the environmental impact of intensive dairy farming. Ryegrass-white clover pastures are characterized by a high crude protein content (above 20% of DM) with high digestibility, producing that a high proportion of dietary N excreted in the urine (Beukes *et al.,* 2014). These issues have increased the interest in evaluating additional forage species and creating more complex pasture mixtures.

The benefits of diverse pastures over monocultures or a simple mix (only two species of plants) includes potentially higher productivity (DM yield), better nutrient retention, more efficient use of available water, reduction of nitrate leaching, and resistance to weed invasion (Sanderson *et al.*, 2004). The higher DM yield results from growth during different seasons that diverse pastures offer. For example, in comparison with a conventional two-species pasture (perennial ryegrass-white clover), a diverse pasture that includes herbs (e.g. chicory or plantain) can grow more vigorously during hot, dry summer periods (Cheng *et al*., 2017).

Legumes have high levels of metabolizable energy. Although a conventional two-species pasture with a high proportion of legumes may have a higher ME/kg DM, the increased yield of multispecies pastures will result in a higher total ME per ha. This can increase the profitability by promoting greater milk-solids production per cow and per ha (Nobilly *et al*., 2013). Soder *et al*., (2006) noted that diverse pastures can have higher yield during dry years and reduced weed invasion, which may allow for an increased stocking rate and result in increased milk production per hectare. Nobilly *et al*., (2013) reported that a diverse pasture that contained perennial ryegrass, tall fescue, prairie grass, alfalfa, red and white clover, plantain and chicory produced on average 16.8 t DM/ha with 202 GJ ME/ha/yr, while the simple pasture containing only perennial ryegrass, tall fescue and white clover produced on average 15.2 t DM/ha with 185 GJ ME/ha/yr. This increase of 1600 kg in diverse pasture reflect a greater DM in summer.

2.2 Pasture feeding value and nutritional requirements of dairy cows

2.2.1 Feed intake and nutritional limitations of pastures

The main cost in any animal production system is the feed, often exceeding 60% of the total production cost. Thus, increasing the utilization of pastures through successfully matching animal demand with forage supply and decreasing off-farm feed purchases will increase profitability (Pembleton *et al.*, 2015). The daily nutritive requirements for small breed cows (e.g. Jersey breed) in mid-lactation producing 20 kg of milk with 4.5% fat and 3.5% protein are 23.6 Mcal and 14.9% CP with an intake of 16.5 kg of DM (NRC, 2001).

Pastures that are commonly used for dairy cows are typically composed of high-quality cool-season forage species. Well-managed pastures with vegetative-stage plants with a high leaf/stem ratio have 18-24% DM, 18-25% CP, 40-50% NDF, and 1.53-1.70 Mcal/kg DM of Net Energy for Lactation (NEL) (Muller & Fales, 1998). The major factor limiting milk production of high producing cows in a grazing system is low pasture dry matter intake (DMI) (Bargo, *et al.* 2003). The DM intake is mainly limited by the high fiber content of forages. This decreases the digestibility of the feed and produces a slow rate of passage in the rumen. Feed accumulating in the rumen distends the rumen and produces a sensation of satiety, although the animal may not have fulfilled its energy and protein requirements. Similarly, high water content of forages can cause a similar effect, decreasing dry matter intake. These pasture characteristics (high levels of fiber, water, and protein) create a gap between the requirements of the animal and the nutrients that the pasture can offer. Improving pasture management can decrease this gap considerably.

The daily pasture intake (kg DM/head/day) in ruminant animals is the product of the intake rate (g DM/min) and the grazing duration (min/day). The intake rate is influenced by the biting rate and the size (mass) of the bite. The grazing duration is influenced by the length (minutes) of meals and the frequency of meals (Orr *et al*., 2001). Factors such as sward density, height and leaf:stem ratio affect intake rate primarily by affecting the bite size (Griggs *et al.*, 2010). The bite mass increases with grass height (until reaching a plateau). However, this relationship depends on the pasture's development stage (vegetative or reproductive), because a greater leaf:stem ratio (e.g. vegetative state) increases the bite rate. The plant species also affects the bite mass; clovers result in a larger bite mass than grasses since the clovers require fewer chews per bite (Ruther *et al*., 2002). Daily intake is also affected by individual animal preference, the physiological state and behavioral requirements of the animal, the environment, and the time of day (Orr *et al*., 2001; Cosgrove & Edwards, 2007).

2.2.2 Milk production from pastures

Compared to conventional confinement systems in which cows are feed with a TMR, Holstein and Jersey cows produced 11.1% less milk on pasture systems. Nevertheless, the economic factors (labor for animal care, manure handling, forage management, and cow culling rates), often make pasture-based systems competitive with confinement systems (White *et al*., 2002). Milk composition is also different; in pasture-based systems: the fat percentage decreases (4.04% vs 4.54% in Jersey; and 3.2% vs 3.75% in Holstein), the protein percentage is equal (3.63% Jersey and 3.49% Holstein); the concentration of CLA is higher, and the ratio of saturated:unsaturated fatty acids is lower. These characteristics have been considered beneficial for human health (Croissant *et al*., 2007).

Milk production (volume and components) varies according to the plant species in the pasture, the nutritive value of the forage offered, the season of the year, and the physiological lactation state of the cow. For example, Pembleton *et al*., (2016) reported that milk production from crossbred-cows in early lactation was 23 L/cow in spatially adjacent monocultures or mixtures of perennial ryegrass, white clover, and plantain, 22 L/cow with white clover and plantain pastures, and 21 L/cow with only perennial ryegrass. Minnee *et al*., (2017) reported that

Holstein-Friesian x Jersey crossbred cows in late lactation had increased milk yield when their perennial ryegrass/white clover diet contained 40% chicory (12.6 kg/cow/day) or 40% plantain (11.7 kg/cow/day) compared to a pasture of only perennial ryegrass and white clover (9.9 kg/cow/day). In contrast, Soder *et al.,* (2006) reported no increase in milk yield from the cows that grazed diverse pastures (orchardgrass, perennial ryegrass, tall fescue, white clover, alfalfa (*Medicago sativa*), red clover, birdsfoot trefoil and chicory) as compared to those grazed simple pasture (orchardgrass and white clover). While Woodward *et al*., (2013) reported that milk production was increased 1.5 kg/cow/day from diverse pastures (perennial ryegrass, white clover, prairie grass (*Bromus wildenowii),* chicory, plantain, and lucerne) compared to a simple pasture (perennial ryegrass and white clover), this increase only occurred during the fall grazing period and it was attributed to an increase in the chicory content of pastures.

Roca-Fernández *et al*., (2016) reported that in Holstein cows, diverse pastures increased milk production 1.1 kg/day and that spatial separation of the plant species increased milk yield 0.8 kg/day and increased intake 1.5 kg DM/day. These effects were observed in all seasons, but were more pronounced in summer, when chicory content increased. Cosgrove and Edwards (2007) also reported an increase in milk yield from cows grazing spatially separated grass-clover strips (19.4 kg/cow/day compared to 15 kg/cow/day with a grass-clover mixture and 14.6 kg/cow/day for the grass monoculture.

2.2.3 Grazing behavior and feed intake manipulation

Pasture preference can be defined as "what the animals select given the minimum physical constraints" (Parson *et al*., 1994). The definition indicates what animals select by their own, with minimal influence of environmental and management factors (Hodgson, 1979). The preference of cattle for pasture species is affected by various factors such as time of day. Cows have a strong preference for legumes in the morning while the proportion of grass selected increases during the day (Cosgrove & Edwards, 2007). Preference of cattle may also be altered by the season, resulting in changes in species availability, access, and palatability. Furthermore, lactating animals show greater preference for legumes (Cave *et al*., 2015). Selective grazing causes a decrease in the presence of the preferred species. For example, selective grazing of clovers in a multispecies pasture can cause a decline proportion of the clovers (Marotti 2004; Chapman *et al*., 2007).

Spatial separation of pasture plants allows each plant species to be managed individually, including weed control and fertilizer management (Pembleton *et al*., 2016). Monoculture strips inside the same pasture can be used to equalize grazing pressure on grass and legume species (minimizing the impact of selective grazing) and increase the legume content in the pasture and diet of grazing animal. With mixed swards, animals need to search in the pasture for their preferred feed (selective grazing). This has a selection cost (a waste of time and energy). This situation can cause the animal to be inefficient when it comes to achieving high rates of forage intake. Animals grazing legume monocultures can increase their intake rate compared with animals grazing grass monocultures; e.g. a sheep in a clover monoculture can have an intake rate of 5.8 g DM min versus 3.5 g DM min in a grass monoculture (Chapman *et al*., 2007). This increased intake can be explained as a lower selection cost (Rutter, 2006).

Related to the establishment pattern of a pasture (separate swards or intermingled), Marotti (2004) reported an increase in milk production by 11% when the grasses and clovers were offered in spatially separated strips (Table 2.1). This increase in milk yield was attributed to a higher legume content of pastures and the decrease in competition between species that exists in the strips. These results are consistent with the finding of Cosgrove and Edwards (2007) where the milk yield from the grass-clover strip was significantly higher than the grass monoculture and the grass-clover mixture (19.4, 14.6, and 15 kg/cow/day, respectively).

Table 2.1. Daily pasture intake and milk production of lactating dairy cows grazing different pasture treatments. Animals were offered a free choice between adjacent monocultures of grass and clover in spatially separated pastures (adapted from Marotti, 2004).

Pasture type	**Intake rate** g DM min^{-1}	**Grazing time** min d^{-1}	**Intake** kg DM head^{-1}	**Milk production** litre head^{-1}
Clover monoculture	53.8	385	20.7	24.2
Grass monoculture	34.0	496	16.9	18.6
Grass/clover mixture	39.6	464	18.4	21.3
Spatially separated monocultures	-	420	20.6	23.2

The main disadvantages of spatial separation are the increased grazing pressure of the legumes (leading to overgrazing and a subsequent decrease in the amount of legume in the diet) and a decrease in the transfer of N which occurs from legumes to the grasses when they are seeded in a mixture, requiring an increase in N fertilizer in the grasses strips (Sharp *et al*., 2012)

2.3 Environmental impacts of dairy farming

2.3.1 Methane (CH_4) emissions

Sustainability is an important concept encompassing environmental responsibility, economic viability, and social acceptability (Ghelichkhan *et al*., 2018). on average, livestock-production contributes 7-18% to the global anthropogenic GHG emissions (Stanley *et al*., 2018) and it is reported that agriculture is the main emitter of nitrous oxide (N_2O) (75%) and second largest emitter of methane (CH_4) (30%) (Rotz *et al.,* 2010). Enteric methane emissions are the largest source of GHG on dairy farms (Rotz, 2018). The U.S. Department of Agriculture reported that a commercial dairy farm with 10,000 dairy cows can produce over 15,000 kg of methane, and 185 kg of nitrous oxide every day (USDA 2011). It is estimated that dairy cows grazing a pasture dominated by perennial ryegrass produce 80-120 kg methane per year per cow; this represents a loss of 10% of metabolizable energy intake (Lee *et al*., 2004)

Methanogenesis is a natural part of the energy metabolism in ruminants. Dry matter intake (DMI) is the major component affecting CH_4 production, as a higher DMI provides a greater intake of fermentable substrate (O'Neill *et al*., 2011). Other components are the size of the animal and the efficiency of the animal to convert feed to product (DeRamus *et al*., 2003). CH_4 production a greenhouse gasses (GHG), and it comes mainly from enteric production by ruminant-livestock. This enteric CH_4 emission can be associated with farming productivity as CH_4 production is related to the rumen microbiome; metabolic processes; and digestion, providing an indirect measurement of efficiency (Hill *et al*., 2016). When comparing animal production systems, it has been suggested that cows release more methane to the environment in pastoral systems compared to grain-supplemented feeding systems, due to higher proportion of fiber and resultant microbial ruminal fermentation. However, recent studies suggest that the carbon sequestration in well-managed grassland systems counter-balances enteric emissions significantly decreasing the overall GHG emissions in pastoral systems (Stanley *et al*., 2018).

Numerous research trials have been conducted to minimize methane emission through diet manipulation. Lee *et al*., (2004) reported that increasing the proportion of white clover in the diet led to a reduction in methane per kg DM eaten, from 21.7 g CH_4/kg DMI in a diet containing 0% white clover, to 18.1 g CH_4/kg DMI in a diet with 60% white clover. Woodward *et al.*, (2004) reported an 18% reduction in methane production per unit DMI in cow grazing birdsfoot trefoil versus ryegrass pasture (19.9 vs 24.2 g/kg DMI, respectively). They suggested this was probably due to the presence of condensed tannin (CT) reducing methanogenesis, although the mechanism by which CT affects rumen function is not well understood.

2.3.2 Nitrogen emissions and leaching

The N content of grass-clover pastures often exceeds the requirements of grazing animals, thus, low protein utilization efficiency is typical in pasture-based production systems. Overfeeding dietary CP increases the amount of N excreted in urine (Castillo *et al*., 2000; Huhtanen *et al.*, 2015). Ghelichkhan *et al.*, (2018) described that the conversion efficiency of N from feed to milk rarely exceeds 30%. It is estimated that 75-85% of the excess protein intake by a dairy cow is excreted in urine and feces and secreted in milk (Munyaneza *et al.*, 2017). The excess N intake can affect the reproductive performance and health of dairy cows (Roy *et al*., 2011). Increased blood or milk urea has been associated with a decrease in reproductive performance of dairy cows (Raboisson, *et al*. 2017, Albaaj *et al.*, 2017) due to the potential toxicity in the uterine environment, changing the uterine pH, and altering uterine secretion activity (Albaaj *et al.*, 2017).

Urea is the metabolic end-product of nitrogen and the main N-containing compound in urine. It is synthesized in the liver from excess ammonia created in the rumen from rumen-degradable proteins, digestible proteins in the small intestine, and the catabolism of amino acids in different parts of the body (Munyaneza *et al.*, 2017). It is estimated that a dairy cow can eliminate between 1.91-5.83 g/L/d of urea-N depending on the high or low content of protein in diet (Misselbrook *et al.*, 2005). Due to its small molecular size and neutral charge, urea freely diffuses between blood and the alveolar epithelial of the mammary gland, thus, there is a strong correlation between urea in blood and milk (Gustafsson and Palmquist 1993; Albaaj *et al.*, 2017; Munyaneza *et al.*, 2017; Raboisson, *et al*. 2017). There is also a positive relationship between the concentration of N in milk, blood, and urine; however, the relationship between milk N (MUN) and N urinary excretion is affected by several factors, including the diurnal dynamics in MUN, which in turn is influenced by the feed intake pattern (Gustafsson & Palmquist, 1993)

In addition to the metabolic cost of producing urea (Totty *et al*., 2013), the excretion of N negatively affects the environment (Castillo *et al.*, 2000). The urinary N excreted by dairy cattle is converted to nitrate (NO3-) in the soil and, due to its mobility in soils, contributes to ground and surface water contamination (Totty *et al.*, 2013). The concentration of N in the urine is determined by the amount of surplus metabolized N to be excreted and the volume and frequency of urination (Hoogendoorn *et al.*, 2010). Manure N can also be transformed to nitrous oxide and ammonia, increasing the greenhouse gas contributions of livestock production (Petersen & Sommer, 2011).

With the increasing concerns about environmental pollution, many research studies have focused on the role of various pasture plant species in reducing the nitrate leached from dairy cow urine depositions (Bryant *et al.*, 2019). The interest in utilization of diverse pasture mixtures as compared to the perennial ryegrass-white clover pastures to reduce environmental impact of dairy farming has been increasing (Nobilly *et al.*, 2013). The novel forages and modern cultivars of recently developed grasses have high content of water-soluble carbohydrates that increase the efficiency of N use, due to the better synchrony of energy and CP in the rumen, which increases the microbial protein synthesis (Totty *et al.*, 2013).

A further approach to reduce the inefficient utilization of N can be achieved through decreasing the release of N in the rumen by decreasing rumen-degradable protein. Phenolic compounds such as condensed tannins present in some forages (e.g. *Lotus* species) bind to dietary protein making it unavailable for rumen degradation, and thus increasing the fecal:urinary N ratio. Fecal N is more stable than urine N (Ghelichkhan *et al.*, 2018), and takes longer to break down, therefore, an increased fecal:urinary N ratio is desirable from an environmental perspective (Totty *et al.*, 2013).

Chapter 3: Herbage yield from diverse and simple pastures sown in mixed or spatially spearated strips

3.1 Introduction

Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are the most commonly grown pasture species on dairy farms in regions where temperate climatic conditions prevail. Perennial ryegrass provides high nutritive value forage for grazing animals and is tolerant of frequent cutting and grazing with excellent biomass recovery (Ostrem, Volden, & Larsen, 2013). White clover is a legume species that is a highly preferred by livestock, provides moderate to high yields, and persists under intensive grazing (Eriksen *et al.*, 2012). The advantages of using these two species together include: large pasture production (7-14 t ha $^{-1}$ per year, varying according to the amount of white clover that the pasture contains); nitrogen (N) transfer from clover to ryegrass; and extension of the grazing season (Elgersma and Schlepers 1997; Roca-Fernández *et al.,* 2016). However, none of these species are tolerant of drought, which limits their suitability range to areas of abundant and seasonally-distributed rainfall or irrigated systems (Nobilly *et al.*, 2013). Approximately, 70% of the annual growth of perennial ryegrass occurs during spring (Rawnsley *et al.*, 2007), leading to a low pasture quantity and quality during summer (Valentine & Kemp, 2007).

In recent years, utilization of alternative forage species in diverse, multispecies pasture mixtures has become a topic of research interest due to potential agronomic and environmental benefits. The major advantages of diverse pasture mixtures over monocultures or simple mixtures include potentially higher productivity, better nutrient retention, more efficient use of available water and resistance to weed invasion (Sanderson *et al.,* 2004). More recently, pasture forbs are being utilized for their positive effects on animal health and the environment, specifically through helping to reduce methane emissions and nitrate leaching problems (Williams *et al.*, 2016; Mangew *et al.,* 2019). Forage species like chicory can increase yield and improve pasture quantity and quality and animal performance during periods when perennial ryegrass growth rates are low (Keith *et al*., 2016; Cheng *et al*., 2017; Minnee *et al*., 2017). Thus, designing diverse pasture mixtures that can improve animal production while reducing the environmental impact of production is receiving more attention.

Designing compatible and persistent pasture mixtures that contain various species with different functional and structural attributes is challenging. Competition for light, energy, and nutrients under selective grazing conditions can lead to the domination of more competitive and less palatable species in the pasture. Several research studies have investigated the effects of spatial separation of pasture species on forage yield, persistence, and animal production (Marotti 2004; Chapman *et al.,* 2008; Roca-Fernández *et al.,* 2016). These studies suggest that spatial separation eliminates the interspecies competition and increases the proportion of more desired, but less competitive plant species in the total planted area. Furthermore, spatial separation of pasture species increased milk yield as the result of increased legume consumption and reduced cost of livestock selection behaviors (Marotti 2004; Cosgrove and Edwards 2007).

The overall objective of the current study was to develop sustainable, high-performing pasture-based dairy production systems where desirable pasture traits for animal performance are maintained at a high level in the diet. The specific objective of the study was to determine annual and seasonal production, pasture growth rates, and botanical composition of forages in simple and diverse pastures planted as mixtures or in spatially separated swards. We hypothesized that multiple species pastures established in spatially separated strips would improve total yield, extend the growing season and have multiple agronomic and animal nutrition benefits such as higher animal feed intake and increased animal production (Marotti, 2004).

3.2 Material and methods

3.2.1 Site, Establishment, and Experimental Design

The study was conducted between 2017 and 2019 at the Oregon State University Dairy Research Farm in Corvallis, Oregon (44° 34’ N, 123° 18’ W 78 m. a.sl.). Soil tests indicated the site had the following conditions: organic matter content, 5.8%; available P (Bray), 113 kg ha^{-1}; Ca, 1772 kg/ha; K, 230 k/ha; Mg, 298 kg/ha; soluble salt, 0.17 dS/m; and soil pH, 5.9. A 7.2-ha paddock was divided into three, 2.4-ha blocks to serve as replicates for the experiment. Each block was divided into 4 subplots (0.6 ha) which were randomly allocated to simple or diverse pasture mixtures, each sown either blended together or as spatially separated adjacent monocultures of grass, herbs, and legumes, giving a total of 12 plots (3 reps of simple monocultures, simple mixed, diverse monocultures, and diverse mixed paddocks). The simple pasture seeding contained perennial ryegrass and white clover. The diverse pasture seedings consisted of perennial ryegrass (*Lolium perenne* L.), festulolium (x *Festulolium braunii*), white clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.), plantain (*Plantago lanceolata* L.) and chicory (*Cichorium intybus* L.) (Table 3.1).

Table 3.1. Sowing rates of pasture mixtures (kg/ha)

Species	**Cultivars**	**Diverse**		**Simple**	
		Mix	**Separated**	**Mix**	**Separated**
Perennial ryegrass	Grasslands Kamo	10	20	16	20
White clover	Seminole	2	5	3	5
Birdsfoot trefoil	Bruce	3	10		
Festulolium	Perun	10	20		
Chicory	Antler	1	5		
Plantain	Boston	3	8		

The four pasture mixtures were sown on 20 October 2017, with 15-cm row spacing in a randomized complete block design with 3 replications with each block as a replicate. In spatially separated plots, monoculture pasture strips were sown in adjacent subplots (0.1 ha) both in simple and diverse pastures (Figure 3.1).

Laneway

<table>
<tr><th>Simple mixed</th><th>Simple separated</th><th>Diverse mixed</th><th>Diverse separated</th></tr>
<tr><td rowspan="6">p. ryegrass-white clover</td><td>p. ryegrass</td><td rowspan="6">p. ryegrass-festulolium-white clover- chicory-plantain- b. trefoil</td><td>b. trefoil</td></tr>
<tr><td>white clover</td><td>chicory</td></tr>
<tr><td>p. ryegrass</td><td>p. ryegrass</td></tr>
<tr><td>white clover</td><td>festulolium</td></tr>
<tr><td>p. ryegrass</td><td>plantain</td></tr>
<tr><td>white clover</td><td>white clover</td></tr>
</table>

Laneway

42 m

143 m

572 m

Figure 3.1. An example pasture block of mixed and spatially separated pasture treatments

Due to a heavy weed and annual ryegrass infestation of the field, the establishment in Block 3 was poor. Therefore, the pasture plots in block 3 was sprayed on 25 March 2018 with glyphosate (at 1.5 kg a.i./ha) to kill all existing vegetation. Following cultivation and seedbed preparation, the pasture plots in Block 3 were re-established on May 20th in 2018. All plots were fertilized with 50 N kg/ha as urea at seeding and 80 N kg/ha in the spring of 2018 (except Block 3). A total of was 214 kg N/ha was applied through dairy effluent in October of 2018. Pastures received full irrigation uniformly as needed with a K-Line (pod) sprinkler system.

3.2.2 Climate

Mean daily low and high temperature and monthly precipitation during the experimental period are shown in Figures 3.2 and 3.3, respectively. Mean daily high temperatures followed a similar trend to the long-term means. However, the mean daily low temperatures were lower than the long-term means in winter 2017 and 2018. In contrast, the daily mean low temperatures were 1.3-5.7 ^{o}C higher than the long-term means during the April-October period in both 2018 and 2019. Corvallis has a long-term mean annual precipitation of 1086 mm. In the 2017-2018 and 2018-2019 growing seasons (October-September), the annual precipitation totals were 893 and 885 mm, respectively. It is of note that the rainfall throughout May 2018 to January 2019 was below average, while in April 2019, the rainfall was more than twice the average, causing a flood in the area.

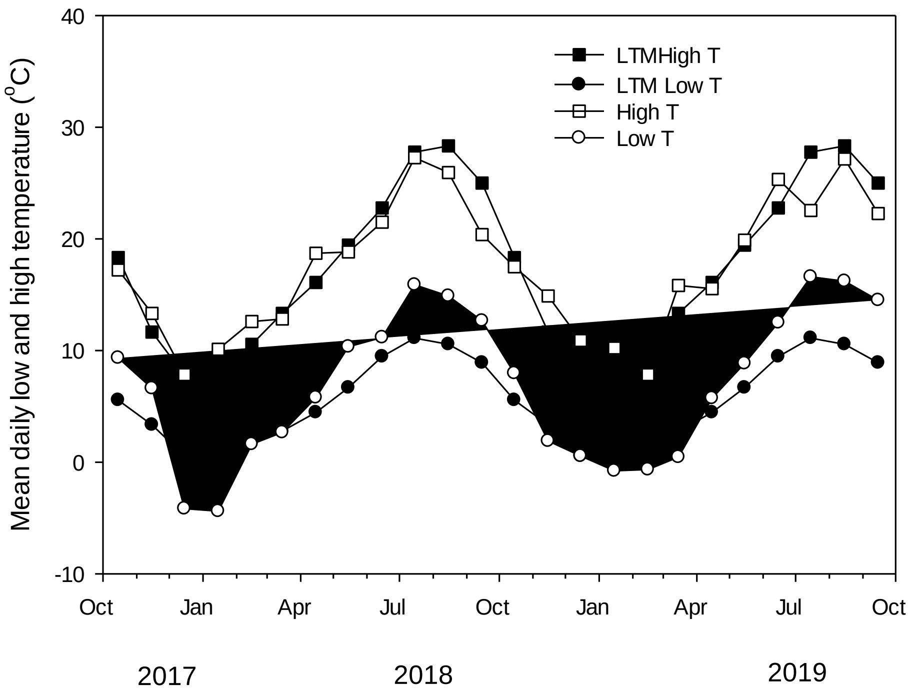

Figure 3.2. Mean monthly low (○) and high air temperatures (□) from 1 October 2017 to 30 October 2019. Long-term means of low (●) and high (■) air temperature are for the period 1980-2010. The meteorological data were collected from the AgriMet Pacific Northwest Region, Bureau of Reclamation

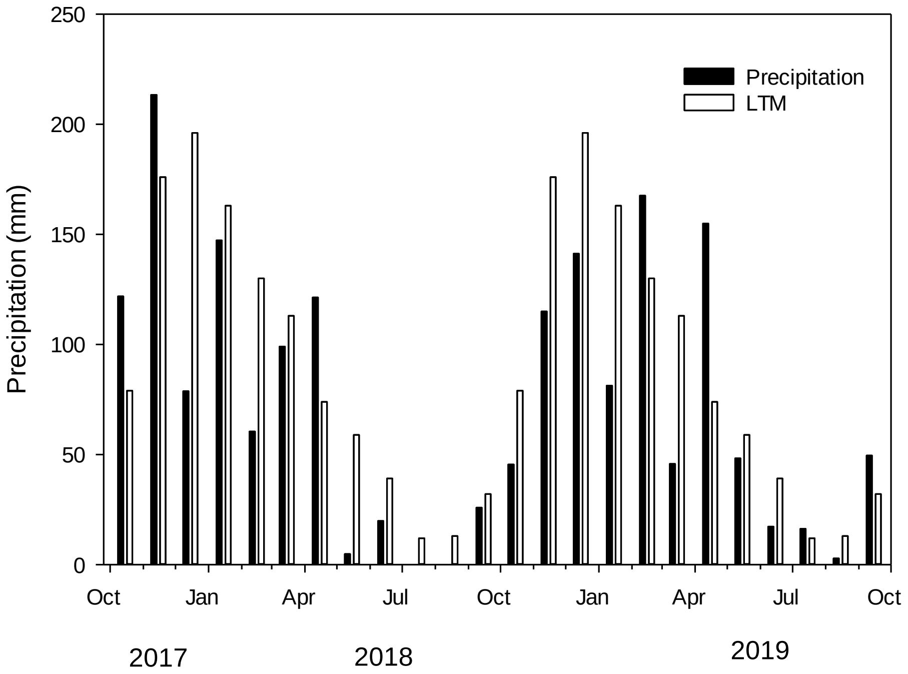

Figure 3.3. Mean monthly rainfall (■) at Corvallis from 1 October 2017 to 30 October 2019. Long-term averages (1980-2010) are shown (□). The meteorological data were collected from the AgriMet Pacific Northwest Region, Bureau of Reclamation

3.2.3 Measurements and Calculations

Pasture dry matter production. Dry matter production (kg/ha) of mixed pastures and each spatially separated monocultures were measured inside 1 m² grazing exclosure cages during active growth in spring, summer, and autumn. Herbage growth was measured from a 0.25 m² quadrat by cutting with electric shears to a stubble height of approximately 3.0 cm. Exclosure cages were placed over a new representative area pre–trimmed to 3.0 cm stubble height at the start of each new growth period. After cutting, cages were relocated to new pre–trimmed sites in each pasture treatment. All herbage from the quadrat cuts was dried in an oven (65 °C) until constant weight.

Botanical composition and pasture growth rates. Quadrat cuts were sub–sampled for sorting into botanical fractions (perennial ryegrass, festulolium, white clover, chicory, plantain, birdsfoot trefoil, weed, and dead material) before they were dried at 65 °C. Herbage growth rates (kg/ha/d) were calculated for each harvest by dividing total DM production by the number of days elapsed since the previous harvest.

Statistical analyses. Herbage DM yield, daily growth rates, and botanical composition were analyzed for each regrowth cycle by ANOVA with replicates as a randomized complete block design for 20 April, 14 May, and 25 June 2018 harvest dates. Total annual DM yield was also analyzed by ANOVA with two replicates. The rest of the data were analyzed by ANOVA with three replicates as a randomized complete block design. The computations were performed using GENSTAT statistical software. Means were separated by Fishers protected L.S.D ($P<0.05$) when ANOVA analyses were declared significant.

3.3 Results

3.3.1 Herbage DM yields

In the 2018-2019 growing season, the total annual dry matter yield of pastures ranged from 9043 kg DM/ha to 11513 kg DM/ha (Table 3.2). Total annual herbage DM production were comparable for simple and diverse pastures. However, mixed pastures tended to produce more ($P<0.07$) DM yield compared to spatially separated pastures. Diverse pastures had higher DM yields than simple pastures during the late spring-early summer period (14 May-25 June 2018). Spatial separation of pastures affected DM yields only during the winter-early spring period in both years. Mixed pastures had greater herbage DM yield both in 2017/2018 and 2018/2019 growing seasons ($P<0.01$). No interaction was detected between diversity and spatial separation for seasonal or total annual DM production in the 2017-2018 growing season ($P>0.05$).

Table 3.2. Seasonal dry matter yields (kg/ha) of diverse and simple pastures sown either as mixed or spatially separated arrangement from October 2017 to June 2019.

Year	Harvest dates	Diverse Mix	Diverse Separated	Simple Mix	Simple Separated	SE	P values D	P values SA	P values D× SA
2018	20 April	2133	904	1941	910	211.9	0.69	0.01	0.67
	14 May	1679	1220	1460	1860	415.1	0.64	0.94	0.37
	25 June	1779	1538	1249	1308	106.7	0.05	0.45	0.25
	13 September	3557	2988	2981	2337	425.3	0.21	0.21	0.96
	22 October	1507	1639	1295	1649	141.2	0.50	0.13	0.46
	3 December	1086	1113	1190	813	177.1	0.75	0.49	0.37
	Total annual	11513	9156	9657	9043	556.6	0.17	0.07	0.21
2019	27 March	2042	1424	1895	1580	129.8	097	0.01	0.28
	8 May	5051	3910	4236	3624	195.0	0.05	0.01	0.25
	17 July	3560	2940	3057	2242	360.2	0.14	0.09	0.79

In the 2018-2019 growing season, DM yield of diverse and simple pastures were comparable between 3 December 2018 and 27 March 2019 (P=0.97). However, mixed pastures had 467 kg DM/ha more herbage yield than spatially separated pastures during the same period (P<0.01). While diverse and mixed pastures had greater production than simple and spatially separated pastures on 8 May, all pastures had similar herbage DM yield on 17 July. However, mixed pastures tended to have greater DM yield than spatially separated pastures during that period (P=0.09).

3.3.2 Pasture growth rates

The effect of pasture diversity and spatial separation on herbage daily growth rates (kg DM/ha) are presented in Table 3.3. Pasture growth rates were slower in the winter period, ranging from 5 to 12 kg DM/ha/d and highest in the spring, ranging from 51 to 78 kg DM/ha/d. In the 2017-2018 growing season, herbage growth rates were affected by pasture diversity in only one harvest period (14 May-25 June) during which diverse pastures had 9 kg DM/ha/d significantly faster growth rates (P <0.05). Spatial arrangement of the pastures affected the growth rates only during the winter-early spring period of the 2017-2018 growing season when mixed pastures had greater growth rates than spatially separated pastures (P <0.01).

Table 3.3. Herbage growth rates (kg/ha/d) from October 2017 to June 2019 of diverse and simple pastures sown either as mixed or spatially separated swards.

Year	Periods	Diverse		Simple		SE	P values		
		Mix	Separated	Mix	Separated		D	SA	D× SA
2017-2018	20 Oct- 20 Apr	12	5	11	5	1.2	0.69	0.01	0.67
	20 Apr-14 May	70	51	61	78	17.3	0.64	0.94	0.37
	14 May-25 June	42	37	30	31	2.5	0.05	0.45	0.25
2018	25 June-13 Sep	44	37	37	30	5.3	0.21	0.21	0.96
	13 Sep-22 Oct	39	42	33	42	3.6	0.50	0.13	0.46
	22 Oct-3 Dec	26	27	28	21	4.2	0.75	0.47	0.39
2018-2019	3 Dec-27 March	32	25	27	23	1.2	0.05	0.01	0.25
2019	27 March-8 May	95	74	80	68	1.3	0.05	0.01	0.25
	8 May-17 July	60	50	52	38	6.1	0.14	0.09	0.79

In the 2018-2019 growing season, diverse pastures grew faster than simple pastures between 3 December 2018 and 27 March 2019 (P<0.05). Similarly, in the early spring period (27 March-8 May), diverse and mixed pastures grew faster than simple and spatially separated pastures. In the late spring and early summer period (8 May-17 July), mixed pastures had 12 kg DM/ha/d greater herbage growth compared to spatially separated pastures (P=0.09) but diverse and simple pastures had similar growth rates during the same period (P= 0.14).

3.3.3 Botanical Composition

Botanical composition of the diverse and simple pastures sown in mixtures or spatially separated swards is presented in Figure 3.4. Both perennial ryegrass and white clover contents of pastures were greater in simple pastures than in diverse pastures. There was an interaction between diversity and spatial arrangement of pastures for white clover content on 14 May 2018 since the difference was only significant in simple mixture pastures (P<0.05). An interaction was also detected between diversity and spatial arrangement of pastures for perennial ryegrass content on 8 May 2019 (P<0.05). The perennial ryegrass content of diverse pastures was similar while simple mixture pastures had greater perennial ryegrass content than spatially separated simple pastures. The amount of plantain and chicory did not differ in relation to spatial separation except on one harvest, in fall of 2018 (P<0.05). Pastures planted in mixtures had greater chicory and plantain content than spatially separated pastures in September and October, respectively ($P<0.05$). Weed and dead material did not differ among pasture treatments. The festulolium contents of mixed and spatially separated pastures were also comparable at each harvest period. However, birdsfoot trefoil content was greater in spatially separated than mixed pasture plantings ($P<0.05$); the difference was only significant on three harvests in the fall of 2018.

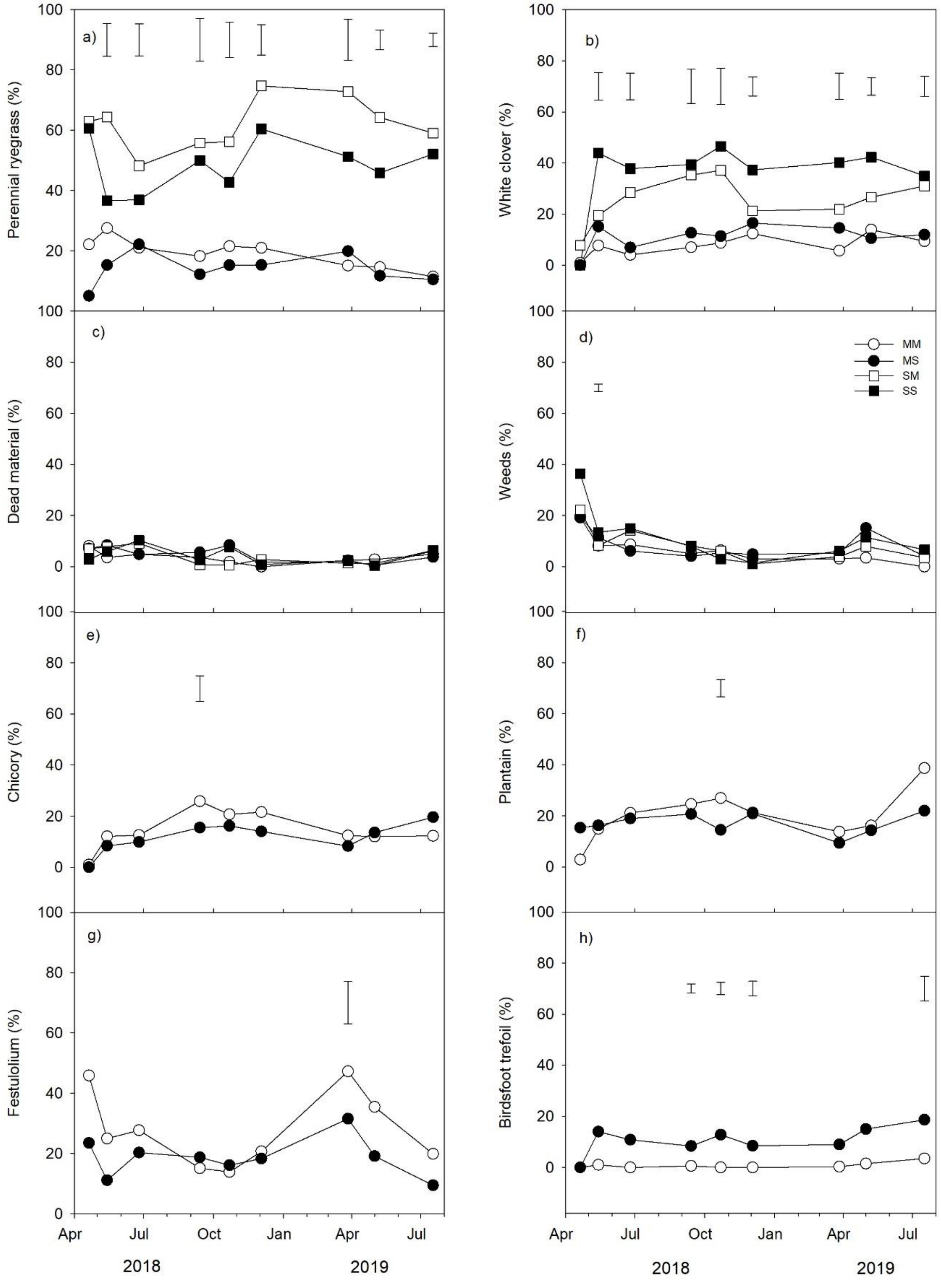

Figure 3.4. Perennial ryegrass (%) (a), white clover (%) (b), dead material (%) (c), broadleaved weeds (%) (d), chicory (%) (e), plantain (%) (f), festulolium (%) (g) and birdsfoot trefoil (%) (h) contents of pastures in 2018 and spring of 2019. Bars represent the LSD above the date when the ANOVA was significant (P<0.05).

3.4 Discussion

The current study focused on the evaluation of DM yield, seasonal growth rates, and botanical composition of simple and diverse pastures sown as a mixture or in spatially separated strips in irrigated conditions. We hypothesized that multiple species pastures established in spatially separated strips would improve total yield, extend the growing season and have multiple agronomic and animal nutrition benefits such as higher animal feed intake and increased animal production (Marotti, 2004).

During the summer of 2018 (June 25th harvest), the diverse pastures produced 0.76 t DM/ha more than the simple pastures, while in mid-spring of 2019 (May 8th harvest) the difference increased to 1.1 t DM/ha. These results are similar to the findings of Nobilly (2015) in New Zealand, where the multispecies pastures during summer produced 0.87 t DM/ha more compared to the simple pasture. Furthermore, the increased herbage yield (by increasing diversity) agrees with the results from Soder *et al*., (2006) in which a two-species pasture (orchardgrass and white clover) yielded less than a six-species pasture (orchardgrass, tall fescue, perennial ryegrass, red clover, birdsfoot trefoil and chicory); 4800 kg/DM ha vs. 7900 kg/DM ha). The improved yield of the multispecies pasture over the simple mixture pasture can be due to the low performance of perennial ryegrass and white clover during the summer period (Cheng *et al*., 2017). White clover can continue growing in summer if irrigation is available, due to the osmatic adjustment to conserve its stolons, and when irrigation is available, the plant can re-grow from stolons (Karsten & MacAdam, 2001). However, perennial ryegrass is not stoloniferous, thus, it does not tolerate drought conditions well. In this experiment, the proportion of plantain and festulolium were the highest during that time (mid-late spring), followed by white clover and chicory. As the temperature increases in spring, plantain begins increasing its yield (Minnee *et al*., 2017; Cheng *et al*., 2017). This explains its increment in spring; while including festulolium in the diverse pasture is beneficial since this grass commonly has a higher yield than perennial ryegrass. Festulolium can yield between 5 and 14.3 t/ha per year and perennial ryegrass between 3 and 9.3 t/ha, depending on the N fertilization levels (Gutmane and Adamovich 2005; Lemeziene *et al*., 2004).

The DM yield was lower under spatially separated sowing arrangement compared to traditional mixed planting of pasture species. This may be lower proportion of the grasses in spatially separated pastures as compared to mix stands. Grasses commonly yield more than legumes, for example Solomon *et al*., (2011) reported that perennial ryegrass monocultures achieve higher yield than a monoculture of white clover and also than a mixed perennial ryegrass-white clover pasture.

A feature of the results was that both diversity and spatial separation of pasture species profoundly affected pasture botanical composition. Spatially separated planting eliminates interspecific competition while allowing plant-specific fertility and weed management practices. The inter- and intra-species competition is particularly critical for slow establishing non-competitive plants. The botanical composition sampling indicated that the sowing arrangement (mixed vs spatially separated) did not alter the proportion of chicory and plantain as evidenced by their similar proportions in both sowing systems. This is mainly because both forb species are quick to germinate and establish in pastures. However, the legume component of pastures, were greatly increased in spatially separated sowing. The increase in the legume content under spatially separated planting arrangement, particularly white clover and birdsfoot trefoil was evident in this research. The increase in legume content of pastures through spatial separation was also reported by Sharp *et al*., (2012). In their research, clover biomass increased from 66.1kg/ha in the mixture to 165 kg/ha in the adjacent monoculture to a perennial ryegrass monoculture. In the present research, the percentage of white clover varied from 25% in the simple mixture to 35% in the simple strip, and from 7% in the multispecies mixture to 12% in the multispecies strip pasture. The percentage of birdsfoot trefoil increased from almost a negligible amount in mixed pastures (1%) to 7% in spatially-separated pastures. This increase was mainly because spatial separation allows for overcoming to main disadvantage of birdsfoot trefoil, its difficulty to establish and poor competitiveness and persistence (MacAdam *et al*., 2006). Spatial separation can also benefit other legumes, as they are not effective at competing for light and moisture in a mixed pasture (legumes and grasses) due to the rapid canopy development that grasses develop (Hayes *et al*., 2016).

3.5 Conclusions

Our findings indicated that diversification of pastures trhough including legume and herb species in mixtures increases the total annual DM yield. In particular, diverse pastures were more productive in summer as compared to simple pastures. Spatial sparation of pasture species benefited non-competitive plant species, like white clover and birdsfoot trefoil, while it did not have a major effect in competitive plants like grasses and herbs. While spatial separation helped increased the proportion of clover content in particular birdsfoot trefoil in spatially separated diverse pastures, the total annual DM production was lower than mixed pastures indicating the main limitation of spatial separation of pastures.

Chapter 4: Milk Production, N partitioning, and Methane Emissions from dairy cows grazing Mixed or Spatially Separated Simple and Multispecies Pastures

4.1 Introduction

High legume content in pastures is desirable for greater forage intake and animal performance. However, competition for resources and selective grazing of livestock usually reduce the legume content of pastures over time, leading to lower performance of livestock. Recent research into this problem has focused on planting pure swards of grass and clover within the same field (spatial separation) as opposed to growing intermingled grass-clover mixtures. Several grazing studies have indicated that spatial separation substantially increased meat and milk yield (Marotti 2004; Solomon *et al*., 2011). The increased animal performance in spatially separated pastures has been attributed to improved diet quality and intake through minimized energy cost of foraging and selection associated with a mixed sward. In this context, the investigation of Chapman *et al*., (2007) reported that when that when grass and legume components of pastures are offered as free choice in a 50:50 area ratio (spatially separated), the feeding value of the pasture is not different from a pure legume monoculture.

While these recent studies have focused on mixed or separated combinations of simple pastures (usually containing two species), the concerted benefits of multispecies pasture mixtures and spatial separation on pasture and livestock production have not been quantitatively evaluated. Diverse pastures containing multiple species have been reported to: help extend the grazing season (Kemp *et al.*, 2010), increase yield (Nobilly *et al.*, 2013), and reduce the reliance on one or two species to meet all the nutritional requirements of livestock. Additionally, certain agronomic and nutritional attributes of pasture plants, such as tolerance to waterlogging and presence of condensed tannins can be better provided in multispecies pastures.

Sustainability is an important concept encompassing environmental responsibility, economic viability, and social acceptability (Ghelichkhan *et al.*, 2018). The environmental concern and consumers' demand for sustainability has affected dairy farms, as they have a role for a sustainable future, due to their contribution to CH_4, CO_2, and N emissions (Rotz, 2018). CH_4 production is one of the main components of the greenhouse gasses (GHG), and the manipulation of dietary components in ruminants can be the most direct and effective ways of mitigation (Thornton & Herrero, 2010). For example, McCaughey *et al.*, (1998) reported that the addition of legumes to a grass pasture can decrease enteric ethane production by 10%. Diverse mixtures have also been proposed as a strategy for improving cow N utilization, in which multispecies pastures containing herbs can reduced the urinary N excretion nearly 50% (Totty *et al*,. 2013).

With the objective of increasing the legume and forb content in the pasture and diet of grazing animals, and minimizing selective grazing, spatially separating species into monoculture strips has been proposed. With mixed swards, animals need to search for their preferred feed (selective grazing). This has a selection cost (waste of time and energy). This can cause gazing animals to be inefficient in acquiring their necessary nutrients. Marotti (2004) showed that milk production can increase by 11% when the typical pasture (grass-clover) is offer as side-by-side strisp of grass and clover, compared to the conventional intermingled grass-clover mixture. This increase can be attribututed to the increment in the amount of clover in the diet. These results are consistent with those of Cosgrove and Edward (2007) in which the milk yield from the grass-clover strip was significantly higher than the grass monoculture and the grass-clover mixture (19.4, 14.6, and 15 kg/cow/day, respectively). In a multispecies pasture, spatial separation can offer the possibility to manage each plant species optimally (Pembleton *et al*., 2016) and not struggle with the herbicide/ fertilizer differences that occurs under multispecies mixed pastures.

Little information is available regarding animal intake, milk yield, and nitrate leaching and methane emission from simple versus diverse forage mixtures in mixed or spatially separated pastures and their persistence. This information would be particularly useful in the prevailing agro-ecological conditions of the northwestern US for enhancing a sustainability farm model. Hence, this study had the objective of investigating milk yield and composition, pasture yield and quality, urea (nitrate) leaching, and methane production from simple and diverse pasture mixtures, grown together or under spatial separation. It is hypothesized that increased diversity and spatial planting arrangement of pasture species with high bioactive compounds would increase intake and milk yield of cows and help reduce environmental effects of pasture- based dairy farming

4.2 Material and Methods

The study was conducted at the Oregon State University Dairy Farm in Corvallis, Oregon (44° 34’ N, 123° 18’ W 256 ft. a.sl.). All procedures were approved by the Institutional Animal Care and Use Committee (ACUP# 5028) prior to the commencement of the experiment. Thirty-six Jersey multiparous and primiparous cows in mid-lactation were used in a randomized complete block design with 9 cows in each treatment. Cows were blocked for age (mean ± s.d.; 3.2 ± 1.3 years), live weight (mean ± s.d.; 492 ± 48 kg) and days in milk (mean ± s.d.; 169 ± 63 d). Each herd of cows contained 2 multiparous and a primiparous cows. Five days prior to start of the experiment, cows grazed on a sacrificial tall fescue paddock together as one herd.

4.2.1 Experimental Design and Grazing Management

A 7.2 ha was used to conduct a 21-day grazing experiment from 3 to 24 April 2019. Dairy cows were offered a dietary treatment of : (**1**) a mixed perennial ryegrass (*Lolium perenne*) + white clover (*Trifolium repens*); (**2**) a spatially segregated perennial ryegrass + white clover; (**3**) a mixed diverse pasture consisting of festulolium (X *Festulolium braunii*), perennial ryegrass, white clover, chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), and birdsfoot trefoil (*Lotus corniculatus*) or; (**4**) a spatially separated diverse pasture consisting of festulolium, perennial ryegrass, white clover, chicory, plantain, and birdsfoot trefoil. Each group of 3 cows were randomly assigned to one of 12, 0.6-ha pastures where they rotationally grazed within the same pasture at the stocking rate of 5 cows/ha. During the 21-d grazing period, the first 14 d were used to adjust the cows to the assigned dietary treatments (transition period), and the last 7 d were used for experimental measurements. Spatially separated adjacent monocultures in both simple and diverse pastures were grazed commonly, as one pasture at the same time. Cows were strip grazed and allocated an estimated 16 kg of DM/cow per day with a post-grazing residual of 1300 kg of DM/ha. Water troughs were moved as needed to ensure ad libitum access to water. The cows were milked twice daily (approximately 0500 and 1800 h) and offered a new pasture allowance after each afternoon milking. All cows received 2 kg DM of rolled grain mix (corn and barley mix 50:50) and 91 g/d/cow mineral mix offered in two equal portions immediately after the morning and afternoon milkings throughout the grazing experiment (acclimation and trial periods). The grain mix contained an average of 9% of crude protein (CP), 12.4% of the neutral detergent fiber (NDF), and 2.3% of ash. The mineral mix consisted of calcium: 17-21 %, phosphorus: 7%, magnesium: 8%, sulfur: 1.65%, selenium: 20-24 ppm, and vitamin A: 200 IU/lb. Body condition of cows (BCS) were scored at each sampling day (d 15, 18 and 21) by two trained, independent evaluators using a five-point BCS scale (1 = thin; 5 = fat).

4.2.2 Apparent Intake and Grazing Behavior

Group feed intake was estimated by determining pre- and post-grazing pasture mass with a rising plate meter (**PM**; Jenquip, Feilding, New Zealand) by collecting 100 measurements in each daily allocation of pasture during the experimental period (last 7 days). A total of 20 rising plate meter readings were taken across the area in each of the spatially separated forage strips. The PM was calibrated by regression against pasture mass by collecting 24 quadrats (each 0.25

m^2, 12 pre-grazing and 12 post-grazing quadrats) per pasture mixture and monoculture. Quadrats were cut to 3 cm residual height with electric hand shears. Apparent group DM intake of cows were calculated from herbage disappearance between pre- and post-grazing herbage and area allocated. Calibration curves for each treatment were generated by fitting a single line through all the data. The calibration curves used were as shown in Table 4.1.

Table 4.1 Pasture plate meter calibrations

Mixtures (kg of DM/ha)	**Pre-grazing**	**Post-grazing**
Diverse mix pastures	88.8 PM + 599.7 (R^2 = 0.71)	141.6 PM + 255.2 (R^2 = 0.68)
Simple mix pastures	168.3 PM – 1822.3 (R^2 = 0.80)	131.1 PM + 103.8 (R^2 = 0.87)
Monocultures (kg of DM/ha)		
Perennial ryegrass	95.7 PM – 138.3 (R^2 = 0.89)	289.7 PM – 3456.3 (R^2 = 0.89)
Festulolium	86.7 PM + 686.8 (R^2 = 0.87)	171.0 PM – 888.0 (R^2 = 0.84)
Chicory	69.1 PM + 112.8 (R^2 = 0.71)	93.8 PM + 127.5 (R^2 = 0.94)
Plantain	74.1 PM – 982.9 (R^2 = 0.79)	143.5 PM + 28.7 (R^2 = 0.78)
White clover	128.8 PM – 60.1 (R^2 = 0.67)	53.5 PM + 899 (R^2 = 0.75)
Birdsfoot trefoil	129.2 PM - 171 (R^2 = 0.65)	60.0 PM + 1042 (R^2 = 0.84)

Random pluck samples were collected from pre-grazing allocations of each pasture to determine nutritive value and botanical composition of forage on offer. A total of 50–75 pluck samples, representative of herbage eaten by cows, were collected randomly by hand across each pasture (with a “zigzag” pattern) at 2 day intervals during the experimental period. Samples were collected within each plot before animals were turned onto fresh pastures. Subsamples were sorted into botanical components and dried at 65°C for 48 h. Botanical composition of samples was then calculated on a dry weight basis. A well-mixed bulk sample was ground in a Wiley mill with a 1-mm stainless steel sieve (Thomas/Wiley, Swedesboro, NJ) for chemical analyses. Samples were analyzed for DM (method 2001.12; AOAC, 2003), ash (method 942.05; AOAC, 2003), and ether extract (method 920.39; AOAC, 2003). The CP concentration of all samples was determined by the Kjeldahl method according to the Association of Official

Analytical Chemists (AOAC, 1990; LECO FP828, MI, USA). Neutral detergent fiber and ADF were assayed according to the methods described by Van Soest *et al*., (1991) using an Ankom$^{200/220}$ Fiber Analyzer (ANKOM Technology Corp., Macedon, NY). Samples were also analysed for their total phenolic and condensed tannins contents. Digestible dry matter content (DMD) was calculated using the following formula DMD=88.9-(0.779×ADF). Total N intake was calculated using the N content (%) of the pasture on offer and the average daily intake (kg) of the 3 treatment groups.

Foraging behavior was scored by visual scanning of each cow at 15-minute intervals from 8 am to 4 pm on April 21 (Orr *et al*., 2005). Each cows' activity was scored for grazing, ruminating, and idling parameters by six observers. Positions of cows in spatially separated pastures were also recorded to determine the grazing time of individual forage strips. Grazing was defined as when a cow was actively eating with their head down. Cows were recorded as idling if they had no specific jaw movements either standing or lying down. Grazing time on mixed pasture stands and each forage monoculture strip, ruminating and idling time were converted from the observation scores and multiplied by 15, assuming the same behavior over the previous 15 minutes. Forage selection ratios were calculated for each pasture monoculture strip (Stuth, 1991). The selection ratios compared the proportion of individual forage monoculture consumed by cows (forage disappearance) to the proportion of that forage monoculture available in pasture on offer. The following formula was used to calculate the selection ratios:

Selection ratio = [(disappearance (DM eaten) of a forage monoculture) / (disappearance (DM eaten) of all forage monocultures)] / [(pre-grazing pasture mass of a forage monoculture)/ (average pre-grazing pasture mass of all forage monocultures)].

4.2.3 Milk Measurements

Daily milk yield measurements were automatically recorded by an AfiMilk system (Kibbutz Afikim, Israel). Milk was adjusted to 4% fat (4% FCM) by using the following equation (NRC, 2001): 4% FCM (L/d) = 0.4 × milk (L/d) + 15.0 × milk fat (kg). Two milk subsamples were collected from each cow after AM and PM milkings on d 0 (baseline), 15, 18, and 21 to determine milk composition. Samples were analyzed commercially (Willamette DHIA Laboratory in Salem, OR) for fat, protein, lactose, somatic cell counts (SCC), and milk urea nitrogen (MUN) by near-infrared spectrophotometry (NIRS). Milk N output was calculated by dividing the milk protein content (%) by 6.38 to give N (%). This was then multiplied by the milk yield (kg/d) to give the total N output in milk.

4.2.4 Blood, Urine, and Fecal Measurements

Immediately after the morning and afternoon milking on d 0 (baseline), 15, 18, and 21, cows were taken into the OSU Dairy free stall barns and restrained for sample collection. Urine samples were collected midstream after manual stimulation of the vulva, acidified below a pH of 3.0 with sulfuric acid to prevent volatilization, and then stored at -20°C until analysis. Feces were collected via manual stimulation or as they defecated and frozen at -20°C until analysis. Blood samples (approximately 20 mL) were collected from the jugular vein. Samples were collected into evacuated tubes (Becton Dickinson Vacutainer Systems Becton Dickinson and Co., Franklin Lakes, NJ) containing lithium heparin for plasma and without additives for serum. After blood collection, tubes with lithium heparin were placed on ice and tubes without additives were kept at room temperature until centrifugation (∼30 min). Serum and plasma were obtained by centrifugation at 1.900 × g for 15 min. Aliquots of serum and plasma were frozen (−20°C) until further analysis. Plasma samples were analyzed for urea concentrations in the laboratory of the Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy, as previously described by Calamari *et al.*, (2016)

Fecal samples were thawed, weighed and dried in an oven at 55°C for 72 h to determine DM. Dry fecal samples were ground to 1 mm and analyzed for percentage. N contents of feces plasma, urine samples, urine NH_3, urine urea, and plasma urea concentrations, were determined by using a N analyser (LECO FP828, MI, USA). Subsamples of urine collected after the morning and afternoon milking from each cow on d 15, 17 and 21 were analysed for concentration of purine derivatives and urea by using HPLC (Agilent 1260 Infinity, Agilent Technologies, Waldbronn, Germany) fitted with a Luna® 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm (cat# 00G-4252-E0, Phenomenex, Torrance, CA) and a SecurityGuard™ cartridges for C18 HPLC columns with 3.2 to 8.0mm internal diameters (cat#AJ0-4287, Phenomenex). Urine samples were diluted 10-fold with double distilled water and filtered using syringe filters and 1ml disposable luer lock syringe (cat# 57022-N04-C and 58901-S, MicroSolv Technology Corporation, Leland, NC). Filtrated diluted samples were inserted into a 1 mL transparent HPLC vials (cat# 82028-402, VWR, Radnor, PA, USA). Urea was determined by fluorescence detection after derivatization using xanthydrol (cat#90-46-0, Alfa Aesar, Tewksbury, MA, USA) and following the gradient III and the automatic HPLC autosampler program of the method of Clark *et al.*, (2007) with modifications. Briefly, the run was 7 min with a full run (up to 12 min) every 10 runs using a blank to clean the column. The column was kept at room temperature (instead of 35ºC). The injection volume after derivatization was 8 µl (instead of 40.5 µl). Furthermore, despite xanthydrol was solubilized in 1-isopropanol as indicated by Clark *et al.*, (2007), it separated quickly decreasing the derivatization of urea. To address the issue, we ran the second point of the standard curve every 10 runs plus we used 3 samples that were added into the sequence every 10 samples and used the data to adjust for the final urea concentration. Quantitation of urea was determined by a 5-points standard curve (4-fold dilution) of purified urea (cat# BDH4602-500G, VWR) prepared in 2.4 pH double-distilled water to match the acidified urine.

Creatinine, uric acid, and allantoin concentration were analyzed using the same column as for the urea following the method described by George *et al.*, (2006). A standard curve constituted of 480 µg/mL of allantoin (cat#97-59-6, Spectrum, New Brunswick, NJ, USA), 120 µg/mL of creatinine (cat#60-27-5, TCI, Portland, OR, USA), and 108 µg/mL of uric acid (cat#69-93-2, Alfa Aesar) diluted in 5-concentrations of 4-fold dilution was used for final quantification.

Microbial N supply was estimated by using equations previously described by Totty *et al*., (2013), Chen (1989), Verbic *et al*., (1990) and Chen *et al*., (1995). The microbial N supply was estimated by the urinary excretion of purine derivatives (PD), allantoin, uric acid, and creatinine and expressed as the following:

PD index = {[total PD (mmol/L)]/creatinine (mmol/L)} × BW0.75.

The PD index was based on the total PD [allantoin (mmol/L) + uric acid (mmol/L)]. Creatinine excretion (mmol/kg of BW0.75) was determined by using the estimated daily urinary volume (L) calculated from the equation by (Pacheco *et al*., 2009). The estimated urinary creatinine excretion (0.9 mmol/kg of BW0.75) was included in the following equation to estimate the daily PD excretion (mmol/kg of BW0.75):

Urinary N excretion (g/d) was estimated using the equation urinary g of N/d = 21.9 (mg/kg) × BW (kg) × [1/ urinary creatinine (mg/kg)] × urine N (g/kg), as described by Pacheco *et al*. (2009).

The estimated urinary creatinine excretion (0.9 mmol/kg of $BW^{0.75}$) was included in the following equation to estimate the daily PD excretion (mmol/kg of $BW^{0.75}$):

Daily excretion of PD (dPD; mmol/kg of $BW^{0.75}$) = PD index × 0.9

The amount of purine absorbed daily was estimated by:

Daily absorbed purine (daP) = [dPD (mmol/kg of $BW^{0.75}$) – 0.385 × $BW^{0.75}$] + 0.85;

Microbial N (g of N/d) supply was calculated with the following equation:

Microbial N (g of N/d) = (daP x 70)/(0.116 × 0.83 × 1000).

4.2.5 Methane (CH_4) Emission Measurement

CH_4 emission of individual cows was determined using the SF_6 tracer method (Johnson *et al*., 2007). A brass permeation tube about 1 cm in diameter and about 4 cm long containing compressed SF_6 gas were targeted to the rumen or reticulum and administered with a bolus gun in cows at the beginning of the trial. The release rate from the permeation tubes was about 1200 ng/min or 2 mg/d. The perm tube was loaded with 600 mg of SF_6 and the release rate was measured gravimetrically for 6 weeks before the perm tube was placed in the cows. A halter containing a collection system comprised of a filtered intake tube, capillary tubing and an evacuated PVC collection canister were fitted to the animal, and the intake tube was positioned near the mouth and nose of the animal. The evacuated canister (< 0.5 mb) had a negative pressure, which drew air continuously for a 24-hour periods through the filter. After the samples were collected, the canister was removed and pressurized with high purity nitrogen gas (N_2). The collected gas was sampled and assayed using a gas chromatograph to determine the concentrations of CH_4 and SF_6. The emission rate of the permeation tube and the ratio of SF_6 to CH_4 in the collection canister were used calculate the enteric emission rate of CH_4 from the animal (Johnson *et al*., 2007). Samples were collected from six replications per treatment (only from two cows in each grazing plot) for six consecutive days (on day to 16 to 21). For the same six days, two ambient air controls were collected in canisters located in a different paddock of the same pastures.

4.2.6 Statistical analyses

All parameters were analyzed by analysis of variance (ANOVA) based on a 2 × 2 factorial model that accounted for the main effects of diversity and spatial separation in a complete randomized design. Treatment means for urine, feces, milk, and blood urea were determined using data collected from individual cows during the experimental periods (AM and PM of d 15, 18, and 21). Averaged across the 3 cows in each plot was used as the experimental unit (pasture plots) rather than individual cows. Herbage and total DMI intakes were estimated as means for the treatment group as cows grazed pastures as small herds (3 cows) together. Covariance analyses were performed for milk yield and components as initial milk yield of cows were not balanced across blocks. Baseline data on urine, feces and blood collected from individual animals were not included in the statistical analyses as treatment effect was not significant. The computations were carried out using GENSTAT statistical software version 18 (VSN International Ltd., Rothampstead, UK) by ANOVA, with pasture treatment (3 levels) and cow block (9 levels) as factors (Payne, Murray, Harding, & Soutar, 2009). Significant differences among treatment means were compared by Fisher's protected least significant difference at $P < 0.05$.

4.3 Results

4.3.1 DMI and Grazing Behavior

Averaged across the treatments, mean pre and post-grazing pasture masses were 3166 and 1609 kg of DM/ha, respectively (Table 4.2). While there was no difference in pre- and post-grazing pasture masses ($P > 0.05$) between diverse and simple pastures, mixed pastures tended to have greater pre-and post-grazing pasture masses than spatially separated ones ($P = 0.06$; $P = 0.09$, respectively). The herbage DMI of cows ranged from 14.8 to 16.1 kg/cow/day, but neither pasture diversity ($P=0.73$) nor spatial separation ($P=0.69$) had a significant effect on DMI. Cows on diverse pastures tended to have higher BCS than those on simple pastures by 0.1 unit.

Table 4.2 Effect of pasture diversity (diverse or simple) and spatial arrangement (mix or separated) on herbage mass, apparent intake and body condition

	Diverse		Simple			P values		
Yield	Mix	Separated	Mix	Separated	SE	D	SA	D×SA
Pre-GPM (kg of DM/ha)	3321	3151	3237	2955	96.3	0.19	0.06	0.58
Post-GPM (kg of DM/ha)	1729	1584	1764	1357	140	0.51	0.09	0.38
Herbage DMI (kg/cow/day)	16.0	15.8	14.8	16.1	1.24	0.73	0.69	0.56
Herbage DMI/kg of BW (g/kg)	3.3	3.2	3.1	3.2	0.28	0.63	0.96	0.67
BCS (unit)	3.0	3.0	2.9	2.9	0.08	0.09	1.00	1.00

D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

The overall means of grazing time, ruminating time and idling time, during the evaluation between morning and evening milking times, were 228 min, 118 min and 134 min, respectively (Table 4.3). Neither pasture diversity nor the spatial arrangement of the pastures had any significant effect on cow foraging behavior.

Table 4.3 Foraging behavior of cows that grazed diverse and simple pastures in mix swards or spatially separated strips

	Diverse		Simple		SE	P values		
Foraging behavior (min)	Mix	Separated	Mix	Separated		D	SA	D×SA
Grazing time	210	233	245	223	13.8	0.37	0.95	0.11
Ruminating time	125	110	118	120	12.1	0.89	0.58	0.49
Idling time	145	137	117	137	14.1	0.32	0.68	0.32
Grazing time of forage strips (min)								
Total grass		95		108				
Total legume		58		115				
Perennial ryegrass		53		108				
White clover		18		115				
Birdsfoot trefoil		40						
Festulolium		42						
Chicory		55						
Plantain		23						
Post-grazing pasture mass								
Perennial ryegrass		1659		1398				
White clover		1414		1271				
Birdsfoot trefoil		1443						
Festulolium		2486						
Chicory		1208						
Plantain		1496						

D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

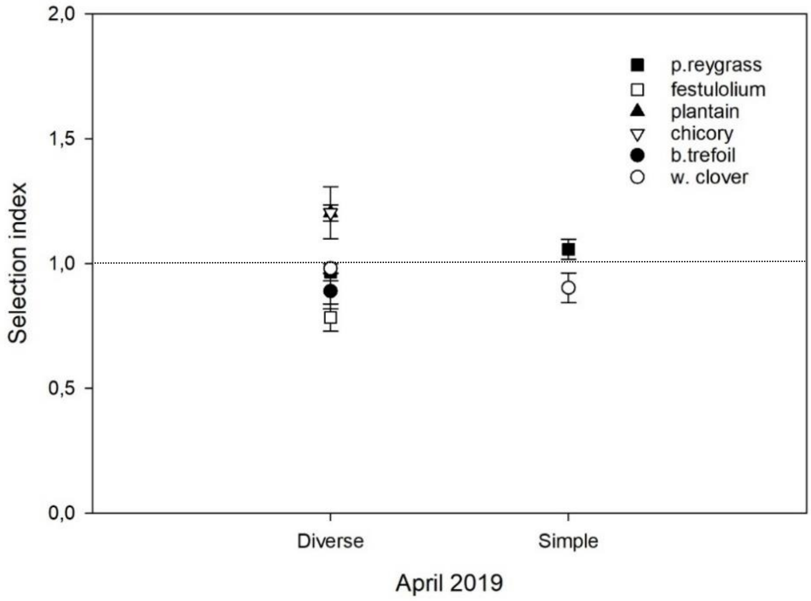

Figure.3. Selection index of individual pasture strips in diverse and simple pastures. Bars represent SED.

The selection index (Figure 4.1) shows a preference for chicory and plantain over other forages (value over 1.0).

4.3.2 Pasture Quality and Botanical Composition

The diversity of pastures did not affect any nutritive value parameters except CP content of pastures (Table 4.4). Overall simple pastures had higher (P <0.01) CP content than diverse pastures (17.2 vs 20.0%). There was also an interaction (P=0.01) between diversity and spatial arrangement of the pastures for herbage CP content. Spatially separated and mixed diverse pastures had similar CP contents, while spatially separated simple pastures had higher CP content than mixed simple pastures by 3.3%. Overall, spatially separated pastures had lower (P< 0.05) NDF contents than mixed pastures (38.9% vs 33.2%). The ADF content of the pastures ranged from 19.0% to 22.5%. Similar to NDF content of pastures, spatially separated pastures tended to have lower (P=0.06) ADF contents than mixed pastures. Increasing pasture diversity (P=0.10) and spatial separation (P=0.10) tended to result in lower EE contents.

Neither diversity nor spatial separation had any effect on ash content of the herbage on offer (P>0.05).

Table 4.4. Nutritive value of diverse and simple pasture in mix swards or spatially separated strips

	Diverse		Simple		SE	P values		
%	Mix	Separated	Mix	Separated		D	SA	D×SA
Ash	9.0	10.2	9.2	8.1	0.70	0.21	0.89	0.15
CP	17.5bc	16.9c	18.3b	21.6a	0.27	0.01	0.01	0.01
ADF	20.6	19.7	22.5	19.0	0.99	0.58	0.06	0.24
NDF	35.9	32.9	41.8	33.4	2.39	0.22	0.05	0.30
EE	2.7	2.4	3.0	2.7	0.14	0.10	0.10	0.86
TP, mg/g	25.2b	83.9a	28.8b	32.7b	6.5	0.05	0.01	0.01
CT, mg/g	8.0	8.6	6.9	7.7	0.47	0.08	0.18	0.79

D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

Botanical composition of pasture on offer revealed significant differences (Table 4.5). Simple pastures had higher (*P*< 0.01) total grass proportions as compared to diverse pastures (68.9% vs 37.4%). Spatial separation resulted in lower grass (*P*< 0.05) but higher legume (*P*< 0.01) contents of pasture on offer. The increase in total legume content by spatial separation was almost 50%. Average white clover content of pastures was higher (*P*< 0.05) in simple than diverse pasture. Spatially separated pastures also tended (*P*= 0.07) to have greater white clover content than mixed pastures. While spatial separation did not affect the proportions of plantain and chicory, festulolium content decreased (*P*< 0.05) through spatial separation of pastures. Birdsfoot trefoil content appeared to be higher (*P*= 0.08) in spatially separated pastures. The broadleaved weed content of pastures did not differ in relation to pasture diversity (*P*=0.55) or spatial separation (*P*= 0.11). However, diverse pastures had higher (*P*< 0.05) dead material contents than simple pastures. It appeared that spatial separation tended to lead to lower dead material content (*P*=0.09).

Table 4.5. Botanical composition of diverse and simple pastures in mix swards or spatially separated strips.

% Component	Diverse		Simple		SE	P values		
	Mix	Separated	Mix	Separated		D	SA	D×SA
Perennial ryegrass	10.3	12.5	75.9	61.9	5.9	0.01	0.35	0.22
White clover	14.5	18.1	20.0	34.1	4.1	0.05	0.07	0.24
Festulolium	34.3	17.8	-	-	2.8	-	0.05	-
Chicory	14.4	17.9	-	-	1.0	-	0.14	-
Plantain	20.7	11.0	-	-	4.2	-	0.24	-
Birdsfoot trefoil	1.0	16.3	-	-	3.4	-	0.08	-
Dead material	4.4	3.0	2.6	0	1.0	0.05	0.09	0.54
Weeds	0.4	3.3	1.6	4.0	1.4	0.55	0.11	0.87
Total legume	15.5	34.4	20.0	34.1	4.5	0.65	0.01	0.61

D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

4.3.3 Milk Production and Composition

Average daily milk yield per cow varied from 23 L/d to 25.7 L/d (Table 4.6), but neither pasture diversity (P =0.15) nor spatial arrangement (P=0.37) had any significant effect on milk yield. However, cows that grazed diverse pastures appeared (P=0.08) to have greater 4% FCM (L/d) yield than those that grazed simple pastures. Milk fat and protein contents were not significantly affected by pasture diversity or spatial separation (all P> 0.05). However, milk produced from diverse pastures appeared (P=0.08) to have higher solid non-fat (SNF) content than those from simple pastures by the diversification of the pasture. The cows that grazed diverse pastures produced 228 g/d more (P<0.05) milk solids and 105 g/d more (P< 0.01) milk protein than those that grazed simple mixture pastures. Milk fat production from diverse and spatially separated pastures tended to be greater than simple and mixed pastures (P=0.08; 0.10, respectively).

Increased pasture plant diversity also led to higher (P<0.05) lactose content in milk, while milk from spatially separated pastures had lower lactose content than mixed pastures by 0.1% (P<0.01). Neither pasture diversity (P=0.68) nor spatial separation (P=0.42) had a significant effect on somatic cell counts (SCC).

Table 4.6 Effect of pasture diversity (diverse or simple) and spatial arrangement (mix or separated) on milk yield and composition.

	Diverse		Simple		SE	P values		
	Mix	Separated	Mix	Separated		D	SA	D×SA
Yield								
Milk Yield (L/d)	23.8	25.7	22.7	23.0	1.16	0.15	0.37	0.49
4% FCM (L/d)	27.9	31.5	25.4	27.4	1.60	0.08	0.13	0.64
Milk solids (g/d)	2234	2386	2095	2070	91.5	0.05	0.51	0.37
Milk fat (g/d)	1228	1413	1090	1215	81.3	0.08	0.10	0.72
Milk protein (g/d)	850	915	788	767	29.4	0.01	0.47	0.19
Component								
Milk fat %	5.2	5.5	4.8	5.3	0.23	0.24	0.15	0.64
Milk protein %	3.6	3.6	3.5	3.4	0.09	0.16	0.53	0.84
SNF, %	9.5	9.3	9.2	9.1	0.10	0.08	0.16	0.96
Lactose, %	4.8	4.7	4.7	4.6	0.03	0.05	0.01	0.23
SCC, $\log_2$	5.4	5.6	5.1	5.5	0.35	0.68	0.42	0.82

SNF= Solid nonfat; SCC= somatic cell count; D= Diversity; SA= spatial arrangement; D×SA= Interaction; SE: Standard Error

4.3.4 Measurements of N in Plasma, Urine, Feces, and Milk

An interaction was detected between diversity and spatial arrangement for dietary N intake of cows (P=0.06) (Table 4.7). Dietary N intake of cows on diverse pastures was similar regardless of the spatial arrangement (mixed or separated). However, cows that grazed spatially separated simple pastures consumed 122 g/d greater N compared to those that grazed mixed simple pastures. Milk urea nitrogen (MUN) content from diverse pastures was 3.6 mg/dl less compared to milk from simple pastures. A tendency of interaction (P=0.06) between pasture diversity and spatial separation was detected for MUN content, as spatial separation had opposite effects on MUN. With the spatial separation, MUN from diverse pastures tended to decrease, while it increased from simple pastures. Milk N output was greater with cows that grazed diverse pastures as compared to those that grazed simple pastures (P< 0.05). However, the excretion of N through milk was not significantly affected by the spatial arrangement of pastures (P=0.29).

Table 4.7 Effect of pasture diversity (diverse or simple) and spatial arrangement (mix or separated) on nitrogen partitioning in dairy cows

Items	Diverse		Simple		SE	P values		
	Mix	Separated	Mix	Separated		D	SA	D×SA
N intake (g N/d)	516	495	502	624	31.5	0.11	0.15	0.06
Urine								
N (%)	0.30	0.29	0.45	0.50	0.022	0.01	0.41	0.29
NH_3 (mmol/L)	2.74	3.21	2.70	3.57	0.39	0.59	0.05	0.50
Urea (mmol/L)	104.0c	98.1c	132.0b	175.7a	5.77	0.01	0.17	0.01
Creatinine (mmol/L)	2.35	2.81	2.69	3.21	0.15	0.06	0.05	0.86
N output (g/d)	139.7	113.6	182.2	176.9	10.16	0.01	0.17	0.34
Feces								
N (%)	3.7	3.6	3.7	3.5	0.04	0.09	0.05	0.62
Ash (%)	19.8	21.5	19.5	21.1	0.77	0.64	0.05	0.93
DM (%)	12.3	11.4	11.0	11.6	0.49	0.33	0.73	0.20
Milk								
Urea N (mg/dl)	10.0	8.6	12.0	13.8	0.69	0.05	0.78	0.06
N output	134.8	144.2	124.7	123.4	4.67	0.05	0.42	0.29
Plasma Urea (mmol/L)	3.48	2.88	4.48	5.22	0.361	0.01	0.84	0.11

D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

4.3.5 Microbial Protein Supply

The urinary concentration of allantoin (*P*=0.08) tended to be greater in cows grazing simple as compared to those grazing diverse pastures. No other PD related parameters were affected by pasture diversity (all *P*>0.05). Urinary uric acid concentration was greater with cows grazing spatially separated pastures than mixed pastures (*P*=0.05). In addition, total PD concentration of cows grazing spatially separated pastures tended to be greater than those grazed mixed pastures (*P*=0.09).

Table 4.8. Urinary concentrations of purine derivatives (PD) from cows that grazed diverse and simple pasture in mix or separated seeding arrangement

Items	Diverse		Simple		SE	P values		
	Mix	Separated	Mix	Separated		D	SA	D×SA
Allantoin, mM	13.34	15.64	15.95	17.05	0.98	0.08	0.13	0.56
Uric acid, mM	0.60	0.84	0.74	0.82	0.05	0.33	0.05	0.21
Total PD, mM	13.79	16.72	16.66	17.85	1.05	0.11	0.09	0.43
Allantoin:creatinine	5.82	5.81	6.07	5.71	0.23	0.76	0.44	0.48
Total PD:creatinine	6.11	6.10	6.35	6.01	0.24	0.76	0.50	0.53
PD index	622.7	622.2	656.0	633.5	23.94	0.30	0.58	0.59
Microbial N, g /d	379.4	379.1	401.0	385.5	13.06	0.32	0.56	0.58

PD= purine derivatives; D: Diversity; SA: spatial arrangement; D×SA: Interaction; SE: Standard Error

4.3.6 Methane emissions

Due insufficient material, methane emissions were only measure in diverse mix, simple mis and simple separations treatments. The daily methane production per cow was 335 g/d, 393 g/d, and 382 g/d for mixed diverse, mixed simple, and spatially separated simple pastures, respectively (Table 4.9). There was no statistical difference for methane emissions of the cows in relation to production of milk and its components (all *P*> 0.05). However, cows that grazed mixed diverse pastures had less (*P*<0.05) methane production per kg of DMI than those grazed mixed and spatially separated simple pastures.

Table 4.9. The effect of pasture type on methane emissions and their relationship to animal productivity

Items	Mixed diverse	Mixed simple	Spatially separated simple	SE	P values
CH_4 (g/d)	335	393	382	20.4	0.22
CH_4 (g/kg of DMI)	18.8b	23.3a	21.2a	0.88	0.05
CH_4 (g kg of milk)	16.2	15.4	16.2	1.33	0.90
CH_4 (g/kg of FCM)	13.9	13.7	13.3	1.22	0.94
CH_4 (g/kg of milk protein)	403	409	378	25.3	0.82
CH_4 (g/kg of milk fat)	318	318	298	29.7	0.86

4.4 Discussion

4.4.1 DM intake and milk yield

The current study investigated the effects of pasture diversity and spatial planting arrangement of pasture species in adjacent strips compared to mixed swards for dry matter intake, milk yield, nitrogen partitioning, and methane emissions of dairy cows. It was hypothesized that increased diversity and spatial planting arrangement of pasture species with high bioactive compounds would increase intake and milk yield of cows and help reduce environmental effects of pasture-based dairy farming. The findings of the current study indicated that increased diversity, particularly through the inclusion of forb and legume species, improved the milk solid and milk protein production and had strong positive effects on FCM and total fat production. Results confirm the benefit of diverse pastures on milk yield previously described (Chapman *et al.*, 2008; Totty *et al.*, 2013; Minnee *et al.*, 2017). Superior milk production was attributed to improvement of pastures quality by inclusion of forb and legume species that have lower neutral detergent fiber (NDF) content as compared to grass species (Pembleton *et al.*, 2015).

In the present study, the improved quality of pastures was more prominent in spatially separated pastures where the legume content of the herbage on offer was 16.5% greater than in mixed pastures. While the diversification of the pasture did not improve pasture quality per se, the positive effect of increased pasture diversity on milk yield was observed. Furthermore, in previous studies it was observed that the superiority of diverse pastures in supporting high animal performance was only seasonally effective (Woodward *et al.*, 2013) and the effect was altered by the daily forage allocation and concentrate supplementation (Soder *et al.*, 2006; Pembleton *et al.*, 2015). In comparison, Soder *et al*., (2006) did not report an increase in milk yield from cows that grazed diverse pastures as compared to those grazed simple pasture possibly due to the high levels of concentrate (40%) supplement in the diet that they used. Similarly, Woodward *et al*., (2013) reported that the increase in milk production from diverse pastures was only apparent during fall grazing period due to an increase in the chicory content of pastures.

In the current study, the effect of diversity on milk components was more obvious on lactose content of the milk aside from the tendency for higher SNF content. It has been reported that condensed tannins (CT) present in birdsfoot trefoil can increase milk protein (Woodward *et al.*, 2000), as they bind to the proteins in the plant and reduce the protein degradation in rumen, increasing the absorption of amino acids in the small intestine. This result also agrees with the findings of Minnee *et al*., (2017) who reported higher milk solids yield due to an increase in the milk protein content of cows that grazed pasture containing chicory and plantain.

Spatial separation of pasture species in adjacent strips did not result in any differences in apparent feed intake and only marginally increased the milk fat yield. The basic argument of the value of spatial separation compared to growing intermingled grass-clover mixtures is increased intake of high quality forages through minimized energy cost of foraging and selection associated with a mixed sward (Moratti, 2004; Solomon *et al*., 2011). Chapman *et al*., (2007) reported that offering legumes and grasses in a 50:50 area ratio as free choice would improve the feeding value of the pastures to a level that a pure legume monoculture would offer. In the present study, although spatially separated pastures offered forages with greater legume contents and slightly better nutritive value as evidenced by lower ADF and NDF values, the difference did not translate into any meaningful intake and yield responses of the grazing cows. However, the lack of difference may be attributed to overall high nutritive quality of pastures regardless of the diversity and sowing arrangements indicating that the chemical and physical composition of pastures were already conducive to maximum feed intake. For example, Pembleton *et al*,. (2016) reported that spatially separated strips and intermingled mixtures of perennial ryegrass, white clover, and plantain pastures had greater milk solid production than perennial ryegrass monoculture and this increased milk production was attributed to increased intake of higher nutritive value pastures, but this affect varied across seasons depending the pasture quality and the physiological conditions of the cows.

Furthermore, in their review papers, Pembleton *et al*,. (2015) stated that the positive response of cows to increased availability of high quality forages in diverse pastures may not be obvious at high forage allocations due to selection opportunity of the grazing cows (Soder *et al.*, 2006; Chapman *et al*., 2008). The lack of difference in DM intake between simple and diverse pasture can also be partly explained by the low DM content in the herbs in the diverse pasture (Minnee *et al.*, 2017) despite the fact that the index selection indicated that cows had a strong preference for chicory and plantain. Similarly, Gregorini *et al*., (2013) reported that cows that were offered chicory and plantain exhibited less rumination time and an increased idling time compared to perennial ryegrass. These results were stronger as the percentage of each herb increased in the diet (from 20% to 60%). The lack of difference in the current study may be related to the temporal grazing pattern of dairy cows as they may have consumed more preferred forages at night after they were offered to fresh pastures. It is also highly probable that at lower pasture qualities than they were offered, the preference may have been skewed more toward pasture forbs.

4.4.2 N metabolism and partitioning

The urinary purine derivate (PD) excretion is a non-invasive method to estimate the duodenal microbial flow (Moorby *et al.*, 2006). This flow is mainly from rumen microbial, therefore, an increase in rumen microbial efficiency will result in increased the PD concentration in urine (Dijkstra *et al.,* 2013). The level of fermentable organic matter in diet is the main factor that determines microbial protein and PD excretion (Earle *et al,*. 1998; Van Duinkerken *et al,*. 2011). The results of the current study indicate that the urine of cows that grazed spatially separated pastures containing forages with lower fiber contents tended to have higher uric acid and total PD contents. In our study we only observed a numerical and non-significant larger N intake in the cows in the simple vs. diverse pasture despite a significant larger CP concentration. It is possible that the high nutritive quality of the pastures in our study provided the rumen bacteria with an abundant amount of carbohydrates that couple with increase of CP concentration. The energy-protein coupling (or nutritional synchrony (Niwinska, 2012)) in the rumen play a major role in the efficiency of the fermentation. A larger microbial protein production is indicative of a proportionally lower amount of rumen undegradable protein.

Rumen-undegradable proteins can be important to increase milk protein synthesis (Grummer *et al.*, 1996)

Excretion of N through in urine, feces and milk has a positive linear relationship with N intake (Mulligan *et al.*, 2004). Overall, urinary N increases at high levels of N intake particularly if energy levels in diet is not matched with higher N intake. This creates a surplus of N, which will not be utilized by the microbes, and therefore, excess amount of N is excreted in urine (Kebreab *et al.*, 2002). The excretion of urea in the present study indicated that cows that grazed simple pastures with higher CP levels had higher levels of urea in plasma, urine, and milk compared with those grazed diverse pastures. This is in line with the findings of Mangew *et al*., (2019) who reported greater urine urea concentrations from cows that grazed perennial ryegrass-white clover pastures (128.7 mmol/L) than those grazed plantain (27.2 mmol/L) and chicory (29.2 mmol/L). Offering a pasture that contains diverse pasture species with high bioactive compounds to dairy cows has potential to reduce the urine N output (Totty *et al*., 2013; Ghelichkhan *et al*., 2018). In the present study, cows that grazed diverse pastures had 40% lower urine N output compared to those that grazed simple pasture mixtures. This result is in line with the findings of Totty *et al.*, (2013) who reported that diverse pasture containing chicory and plantain decreased urinary N by 50%. Decreased urinary N output and increased milk N output under the treatments with low CP (diverse pasture), suggest a shift in the excretion of N (Totty *et al.*, 2013). This situation can be explained by the ratio of water soluble carbohydrates to crude protein in the diet, and a possible effect of the secondary metabolites present in the herbs.

4.4.3 Methane (CH_4) emissions

Similar to the findings on N partitioning, some positive effects of diverse pastures was also observed on CH_4 production. Although the daily total methane production per cow was not altered by the pasture type, cows that grazed diverse pastures had 20% lower CH_4 production per kg of DMI compared with cows that grazed simple pastures. These findings are in agreement with the results reported by (Woodward *et al.,* 2004) where Holstein-Friesian cows that grazed birdsfoot trefoil had a 17% lower CH_4 emission per unit of DMI compared with cows that grazed perennial ryegrass. The decrease in methane production can be attributed to the presence of secondary metabolites that chicory and birdsfoot trefoil contain (Woodward *et al.,* 2004; Williams *et al.,* 2016). Although, the mechanism of condensed tannins on methane reduction effect still require further scientific studies, positive effects of condensed tannins on methane productions have been reported (Woodward *et al.,* 2004). Condensed tannins, depending on their source and levels in diet affect methane-producing bacteria in the rumen and possibly reduce the methanogenesis through several hypothetical mechanism, being the most likely a sequestration of hydrogens by catechin (Bodas *et al.,* 2012; Naumann *et al.,* 2017). It was of note that cows that grazed spatially separated or mixed simple pastures had similar CH_4 production, although the spatially separated pastures had 14.1% higher white clover content. In line with this finding, (Wilson, 2020) reported comparable CH_4 emissions from cows that grazed grass (325 CH_4 g/d/cow) or legume-based (348 CH_4 g/d/cow) pastures in the late spring-early summer period. They also noted that the methane emissions of cows that grazed forb-based (278 CH_4 g/d/cow) pastures appeared to be lower. In contrast, Lee *et al,*. (2004) reported that increasing white clover content in pastures resulted in lower reductions in methane per kg DM eaten, although total daily CH_4 emissions from pastures with higher legume contents increased. The lower methane per kg DM eaten was a direct result of increased intake of pastures with higher legume content. It is highly probable that, the legume content may have a more profound effect with lower quality pastures, as increased intake and high forage digestibility leads to lower methane emissions though improving the overall production efficiency (Hegarty 1999; Hristov *et al.,* 2013)

4.5 Conclusions

This work is a new approach in the use of legumes, grasses, and herbs in temperate pastures. It offers new possibilities for the incorporation of plant material with novel nutritional characteristics, but weak agronomic attributes such as slow establishment, poor competition or short life cycle, into livestock grazing systems. The diversity of the pasture maintains milk yield compared to a simple pasture, while it allows an increase in milk solids, through an increase in milk protein, and an environmental and economic benefit from improved control over methane emissions and urea excretion in the diverse pasture system. Spatial arrangement of forage species can increase the pasture quality due to an increase in low competitive plant species (legumes), but this improvement did not affect dairy cow performance.

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