Oat, pea, and canola intercropping: An investigation of the agronomic benefits and underlying biological mechanisms of a multi-crop forage system

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Soil Science

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Abstract

Oat, pea, and canola intercropping can provide benefits in terms of beef cattle forage as it can provide stable biomass and nutritional yield. Two underlying mechanisms were examined as potential explanations to its stability: 1) plant water use efficiency, and 2) arbuscular mycorrhizal fungi (AMF) community composition. This two-year project was a two-factor complete block design located at the Peace Country Beef and Forage Association research farm (Fairview, Alberta), where forage biomass, nutritive indicators, normalized difference vegetation index (NDVI), photosystem II photosynthetic efficiency (P[II]), water use, and mycorrhizal communities were sampled yearly. The two factors present in this project are intercropping systems and nitrogen (N) fertilizer addition.

In Chapter 1, basic concepts that pertain to this project are revisited through previous studies with similar experimental designs. Chapter 2 provides insights to forage biomass and quality obtained from the project as a study of the benefits of intercropping as beef cattle forage. In general, intercropping was found have very stable biomass yield, as well as forage quality. The effect of N fertilizer depended heavily on the cropping system. Pea-canola intercropping performs better than pea alone or canola alone in terms of crude protein and certain mineral contents. Chapter 3 provides insights to water use efficiency (WUE) and water uptake, where pea-canola intercropping demonstrated promising increase in WUE. These results also showed that increased water uptake does not consistently translate to improved WUE. Additionally, the effects of N fertilizer were only significant in a wetter growing season. In Chapter 4, AMF communities were observed to increase in diversity when intercropping oat and pea, and the effects of N fertilizer was present in the drier year when the difference in AMF abundance was significantly different. Key genera were identified for the intercropping system examined and

provided insight to how it could be further studied. Chapter 5 summarizes the project and provides potential directions to further the results found in this project.

Preface

Chapter 2 of this thesis will be submitted as Lee, A., Omokanye, A., and Hernandez-Ramirez, G., "Intercropping oats with peas and canola can stabilize forage nutritive quality, and increase forage biomass productivity" to *Crop, Forage, and Turfgrass Management*. I was responsible for the data collection and analysis as well as the manuscript composition. A. Omokanye assisted with the data collection and was the supervisory author that was also involved with concept formation and manuscript composition. G. Hernandez-Ramirez was involved with concept formation and manuscript composition.

Chapter 3 of this thesis has been submitted as Lee, A., Omokanye, A., and Hernandez-Ramirez, G., "Oat-pea and pea-canola intercroppings with and without nitrogen fertilization alter biomass and crude protein water use-efficiencies" to *Agricultural Water Management*. I was responsible for the data collection and analysis as well as the manuscript composition. A. Omokanye assisted with the data collection and was involved with concept formation and manuscript composition. G. Hernandez-Ramirez was the supervisory author and was involved with concept formation and manuscript composition.

Chapter 4 of this thesis will be submitted as Lee, A., Gorzelak, M., Neuberger, P., Omokanye, A., and Hernandez-Ramirez, G. "Identifying key mycorrhizal genus in oat-pea intercropping: How mycorrhizal genera can help determine stressed plants and how N fertilizer affects mycorrhizal abundance"**.** I was responsible for the data collection and analysis as well as the manuscript composition. M. Gorzelak provided the methodology and resources for data collection and contributed to manuscript edits. P. Neuberger assisted with the data collection and contributed to manuscript edits. K. Kim assisted with the data analysis and contributed to manuscript edits. A. Omokanye was involved with concept formation and manuscript composition. G. Hernandez-Ramirez was the supervisory author and was involved with concept formation and manuscript composition.

Dedication

I remember it was the end of a growing season, the sun was setting, and we were just standing in from of a plot of intercropped corn that we were going to harvest very soon. I said something like “the corn looks like it grows just as well with and without soybean in between, doesn’t it?” and the reply I got was something alongside the lines of “it does, doesn’t it? Isn’t that fascinating?”. Yes, it was indeed fascinating. In fact, so fascinating I started asking myself, and at the time, Akim, “what if we used crops that producers use a lot up here to do intercropping instead? I think I know a professor that might be interested”. While I do thank my parents for their continuous support to let me have free will to do whatever I thought would make me happiest, I could have never been here if not for the continuous encouragement from my friend and supervisor, Akim and Guillermo, to try to complete a master’s degree. Thank you so much for this once in a lifetime experience, and I hope that from here on out, I’ll have even more opportunities to work with wonderful people like you.

Acknowledgements

I would like to thank the team at Peace Country Beef and Forage Association (Liisa Jeffrey, Kaitlyn McLachlan, Johanna Murray, Buthaina Al-Maqtari), as well as colleagues from the Sustainable Land Ecosystems lab at the University of Alberta (Keunbae Kim, Kris Guenette, Erin Daly, Keifer Klimchuk) for the continued support over the course of this project. I would also like to thank Monika Gorzelak and Patrick Neuberger for the technical support specifically in the mycorrhizal part of this project. This research was financially supported by the Canadian Agricultural Partnership (CAP) - Environmental Stewardship and Climate Change Group Program (ESP-048569).

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Chapter 1: Introduction

Agriculture is expected to intensify as the human population grows. While intensifying agriculture often refers to an increase in fertilizer use, irrigation, and land use, it is becoming an increasingly unfeasible option. The increase in fertilizer use is accompanied by diminishing returns (Liang et al. 2021; Qiang et al. 2020; Hendricks et al. 2019). As the plant reaches its maximum yielding threshold, the gain per kilogram of fertilizer decreases, salinity content increases, and nutrient leaching is more prominent. The consequence of overfertilization becomes a cost to mitigate the negative effects it produces. Besides a decrease in yield, overfertilization leaves plants more susceptible to undesirable conditions, such as cold damage or lodging (Russell et al. 2006). Irrigation is not always accessible. For example, in Alberta, Canada, irrigation can be limited by the Water Act to comply with conservation efforts (Province of Alberta 2017). While clearcutting could increase production yields as there would be more land for growing, lands suitable for agricultural transformation is limited. In the situation where intensification options become limited, we become obligated to explore new options. One proposed option is called intercropping, where more than one crop is grown on a given piece of land.

Intercropping is a common practice used throughout Asia, Europe, and Africa, and is often used because of its overyielding effects (Gao et al. 2009; Chai et al. 2014; Ren et al. 2017). The speculated reason for the overyielding effect is that there is plant competition and that the intercropped plants may be using different nutritive sources. However, there is limited research on this topic. In this chapter, I will explore the different articles that explain the potential reasons for the overyielding effects from intercropping, as well as the yield effects for cattle forage use. The subjects include species selection, water use efficiency, and mycorrhizal diversity.

1.1 What is Intercropping?

Intercropping, as mentioned previously, is a common practice used across many countries to alleviate the pressure of agricultural intensification. This practice outdates modern practices of fertilizing and genetic alteration (Eames-Sheavly 1993). In the Iroquois legend, the three sisters (corn, beans, and squash) were useful in different ways, and worked best when together. Applied to modern agricultural production, we find that there are more ways to pair crops together than just the Three Sisters method. Some intercropping practices include growing maize with peas, maize with soybean, maize with wheat, oats with peas, as well as some vegetable mixtures. Besides plant mixtures, plant growing arrangements also dictates how well intercropping will be affected (Du et al. 2016; Caviglia et al. 2004). In intercropping, one could choose to grow plants with similar growing seasons and harvest altogether or have plants with different growing length and staggered harvest times. By removing some crops early, the residue from the removed crop becomes a nutrient source of the remaining crops (Yang et al. 2018). Although it could lead to a reduced yield for the crop harvested early, the improved yield of the later crop generally outweighs the loss from intercropping, thus making this method a viable cropping solution. Plant spacing can also affect the effectiveness of the intercropping method (Rashwan and Zen El-Dein 2017; Zhang et al. 2008; Ren et al. 2016; Ren et al. 2017; Singh et al. 2011). The intercropped plants could be mixed in the same row (mixed row), or alternate between one crop then the other, where the alternating method could range from 1:1, 2:1, 2:2 and so on (alternate row). Depending on the plant requirements and competitiveness, how the plants are seeded could determine the effectiveness of intercropping for each plot. As such, intercropping is a cropping system that relies on balancing crop selection, crop spacing, and crop harvest methods while catering to the location environment to achieve the most optimal yield in a season.

1.2 Intercropping for improved plant growth and forage quality

Regarding plant physiology, one would imagine competition between plants would increase plant height and root length to compete better with the opposing plant. However, there has not been much found in terms of taller plants, or plants with longer roots. Instead, research has found that by placing some plants together, peas and oats for example, it reduces lodging frequency (Kontturi et al. 2011). As reduced lodging increases harvestability and reduces diseases, oat-pea intercropping could promote increased yield. There is speculation that different root systems can complement each other when intercropped. Qin et al. (2018) explains that since the wheat root system is longer, it can access stored water when inter-seeded into an irrigated maize plot mid-season (2018). This increase in root diversity per layer could lead to better water use efficiency (WUE) as well as better nutrient use efficiency (NUE). While it seems effective in maize-soybean and wheat-cotton relay intercropping systems (Yong et al. 2018; Zhang et al. 2008), improved NUE was not observed in an oat-pea intercropping system. As mentioned by Zhang et al. (2008), proper timing and management is necessary to achieve higher NUE. Similarly, WUE is also influenced by proper crop management (Wang et al. 2015; Fan et al. 2013; Chai et al. 2014), where intercropping performs better than single cropping under moisture stressed scenarios (reduced irrigation). In conclusion, the main physiological benefits of intercropping are the mutualistic interaction between intercropped plant varieties, where, despite the presence of competition of nutrients, the plants are effectively cooperating with each other to attain optimal growth, which in turn develops into higher yielding values.

To provide proper nutrition to facilitate sufficient energy for maintenance, gain, and lactation, cattle feed must be properly managed. Intercropping can give a competitive edge in terms of producing more yield, and it can also serve as a nutritional balancing method (Bacchi et al. 2021; Kamalongo and Cannon 2020; Atis and Acikalin 2020). When growing monocrops such as cereals or legumes, a potential issue occurs where the yield of cereals is poor in terms of crude protein (CP) and the yield of legumes is low in terms of biomass. The combination of a legume and cereal crop can balance crude protein and biomass yields, albeit having a slight disadvantage compared to the monocropped system (Omokanye et al. 2020; Bacchi et al. 2021). The protein requirement for a cow that is between mid-pregnancy and lactating and cattle that is growing to finishing requires 7-11% CP and 12-14% CP, respectively (Omokanye et al. 2020). By mixing the optimal intercropping ratio, dry matter yield and CP can be matched to the cattle requirements (NASEM 2016).

1.3 How is Mycorrhiza Involved in Increasing Yield?

Arbuscular Mycorrhizal (AM) symbiosis serves many purposes in the soil ecosystem. It can, for example, aid in increasing drought tolerance (Nouri et al., 2020), disease tolerance (Pawlowski and Hartman, 2020), and improved nutritional access for growth (Gashgari et al., 2020). However, AM symbiosis does not simply function under the presence of the mycorrhizal fungi itself. As shown in the work done by Koziol, Crews, and Bever (2020) in a prairie restoration effort, native mycorrhizal fungi encouraged native plant abundance, richness, and community diversity, while commercial mycorrhizal products were unable to achieve the same results. It is therefore imperative to understand the necessary conditions in which AM symbiosis provides a benefit to an ecosystem.

To visualize the transport of the nutrients to the plants, many studies used containers with root impeding barriers to observe hyphal infection and nutrient transport through the barriers (Argüello et al., 2016; Hodge, Fitter, and Díaz, 2010; Leigh, Hodge, and Fitter, 2009). As roots cannot access the compartment with excess nutrients, it is assumed that the hyphae are translocating the nutrients from that compartment to the compartment with the plant roots. To further confirm the findings, some studies have used tracer elements such as isotopic P, N, and C to observe the uptake and the transfer of these nutrients (Ren et al., 2012; Argüello et al., 2016). The conclusion from the results found was plants of the same species compared to its non-mycorrhizal counterpart does in fact uptake more nutrients. In the case of watermelon and rice intercropping, AM colonization was observed to transfer root exudates from the rice to the watermelon, which was then taken up by the watermelon (Ren et al., 2012). Furthermore, an increase in P uptake could be observed in the rice plant in the intercropped treatments compared to the monocropped treatments, indicating that the exudation of the rice roots may be promoting mycelial transfer of plant available P.

Beyond mobilization of P, there has been studies demonstrating the ability of Arbuscular Mycorrhizal symbiosis aiding in the uptake of N (Leigh, Hodge, and Fitter, 2009; Hodge, Fitter, and Díaz, 2010). Past reviews have indicated the possibility of N uptake through AM symbiosis (George, Marschner, and Jakobsen, 1995), and recent reviews have confirmed it. Recent reviews have also discussed how the translocation of N occurs (Hodge and Storer, 2015). In an AM symbiotic relationship, the mycorrhizal fungi can transfer an immense amount of N to the host plant; up to 30% of the total plant N was contributed from the AM symbiosis (George, Marchner, and Jakobsen, 1995). Organic material is seen to be taken up through AM fungi and transferred to the plant and contributes roughly 5% of total plant N (Leigh, Hodge and Fitter, 2009; Hodge, Fitter, and Díaz, 2010). While it does not increase total plant N capture (Leigh, Hodge and Fitter, 2009), it does express the possibility that mycorrhizal symbiosis provides an alternative source of N when the plot has low available N presence (Hodge, Fitter, and Díaz, 2010). The experiments also show the importance of fungal diversity as certain fungal symbiosis provides more benefits over the other, as in the case of *Glomus intradices* versus *Glomus hoi*, where *G. intradices* outperforms *G. hoi* in nitrogen transfer to plants (Leigh, Hodge and Fitter, 2009).

Mycorrhizae can also increase the uptake of micronutrients, most notably the uptake of copper, zinc, and iron (Liu et al., 2000). Plants are observed to have higher micronutrient content when there is an increase in density and colonization of AM fungi (Ryan and Angus, 2002; Liu et al., 2000). As the micronutrients are generally immobile, uptake from roots often create "depleted zones" around the rhizosphere (Liu et al., 2000). The benefit of the AM symbiosis is the capability of the fungal transport of the nutrients closer to the plant for uptake, therefore bypassing the "depleted zone" limitation (Liu et al., 2000). The benefits, however, are constrained under certain conditions, such as competition and nutrient limitation, and may not produce significant benefits if the conditions are not met (Ryan and Angus, 2002; Liu et al., 2000). It is therefore important to explore the conditions that maximize AM symbiosis efficiency by understanding how mycorrhiza react under different conditions.

1.4 Diverse Mycorrhiza with Diverse Mycorrhizal Functions

There is an astronomical number of mycorrhizal species, including many species that are undocumented. In many studies where fungicides were used, the decrease in AM fungi led to a change in the community structure, where an increase or decrease in plant diversity can be observed depending on whether the plant community is dependent on mycorrhizae or not (Gollotte, van Tuinen, and Atkinson, 2004). This ecological behaviour shows that mycorrhizal diversity plays a significant role in the soil ecosystem, and potentially a mediator in plant diversity. While it is not clear whether the mycorrhiza is required for the plant presence or vice versa, the symbiotic diversity is plant dependent (Gollotte, van Tuinen, and Aktinson, 2004; Gorzelak, Pickles, and Hart, 2017). Meaning, mycorrhizal symbiotic diversity with each plant differs by species. This mutualistic relationship could be explained by the benefits plants receive from the relationship, as different fungi may produce different benefits, and some benefits more than others (Leigh, Hodge and Fitter, 2009). The diverse pool of fungi may synthesize better with a certain plant over another and proliferate, causing a dynamic shift in species diversity. Thus, the purpose of diversity in mycorrhizal fungi helps shape the soil community to fit the plant's needs. As such, the implementation of intercropping could harness the diverse benefits that mycorrhizal symbiosis can provide.

Plant age does not change the diversity of mycorrhizal colonizers, but location does (Gorzelak, Pickles, and Hart, 2017). Furthermore, not only does the location dictate the type of colonizers that mutualize with plants, but the plant hosts also dictate this action (Pickles et al., 2015). The phenomenon occurs as different plant species have different nutritional requirements, as well as different resource acquisition strategies in different situations (Pickles et al., 2015). One key strategy that influences the fungi in AM symbiosis in plants is root exudation, where the plant secretes nutritional substances into the soil. Root exudation has shown to maintain fungal communities in the soil, and different plant exudation can determine the structure of the community (Broeckling et al., 2008). This could explain how a plant community can change over time in diverse scenarios, such as forest ecosystems, where successional fungal communities can be observed in individual plant species as it is succeeded by younger plants (Hart et al., 2014). Additionally, this is a subject that can be further expanded into an agricultural setting, as succession in agriculture happens as the plant grown tends to be annual, thus observing the change in mycorrhizal diversity could provide insight into best management practices to improve production yield.

1.5 Applications to the Peace Region, Alberta, Canada

While the benefit of intercropping is well understood, the system that provides the most ideal benefits is highly dictated by geographic climate and conditions. By selecting different plants for intercropping, different arrangement methods must be applied to cater to optimizing the yield performance. Furthermore, the selection is highly based on the growing season of the location, as well as soil type. With the understanding of the importance of mycorrhizal diversity, selecting crops based on the available mycorrhizae in the soil should become an essential practice as well. That said, the knowledge of which mycorrhizal species work best with which plant type becomes a key tool in the future that has yet to be explored. Plant usage, plant spatial arrangement, and best management practices can be determined with improved understanding of plant-mycorrhizae symbiosis. This is possible by creating a method to determine mycorrhizal content and the complementary pairs.

In the following chapters, I will apply the concept of intercropping explored here to the Peace region of Alberta, Canada, to confirm and expand on the benefits that it can provide. Firstly, I will confirm that overyielding is in fact a phenomenon that occurs in an intercropped system. This will include examining the yield quality and quantity, as well as WUE and NUE. To expand on the underlying cause overyielding, I hope to use metrics such as NDVI, plant height, photosynthetic activity, and mycorrhizal density and diversity. The purpose of this research is to increase awareness of alternative production methods within North America and provide more evidence to why intercropping should be considered to reduce input costs.

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Chapter 2: Intercropping oats with peas and canola can stabilize forage nutritive quality, and increase forage biomass productivity

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2.1 Abstract

Forage intercropping can increase forage production while maintaining good forage nutritional quality. This study examined the effects intercropping oats (O), peas (P), and canola (C) at varied nitrogen fertilizer rates of 0 (0N), 50 (50N), 75 (75N), and 100 (100N) kg N ha^{-1} on biomass productivity and nutritive value indicators for beef cattle in 2019 and 2020 growing seasons. OC at 0N produced the overall highest yield in 2019 at 11.6 Mg ha^{-1}, higher than O at 75N by 1.30 Mg ha^{-1}. In 2020, O at 0N produced the overall highest yield at 9.09 Mg ha^{-1}, higher than OP at 100N by 0.2 Mg ha^{-1}. Forage nutritive indicators were not impacted by the interaction of intercropping with N rate within each study years. PC consistently had higher crude protein (CP) content than the rest of the intercropping systems (13.6% CP in 2019, 15.4% CP in 2020), while also producing high forage biomass. This was also reflected by the better nitrogen utilization efficiency (NUtE) of PC over P and C alone in both 2019 and 2020. While none of the assessed intercropping treatments, including the control, met the optimal mineral requirements for beef cattle, triple intercropping (OPC) had the most stable forage nutritive quality across all evaluated options. By adding canola to intercropping systems, calcium content frequently showed a

noticeable increase. In sum, including canola in intercropping systems optimized forage production. For high forage-based productivity cropping systems, PC intercropping is recommended over P or C alone. For achieving stable yield and nutritional quality of annual forage crops under soil and climate conditions comparable as in our study, OPC is recommended. Future research can focus on evaluating beneficial seeding rates of canola in intercropping to further optimize forage yield and quality.

2.2 Introduction

Agriculture needs to cater to the needs of the growing world population. In addition to sourcing food, croplands also deliver biomass for livestock feed and biofuel production (FAO/OECD 2018). In Western Canada alone, there is a large concentration of beef cattle production (Statistics Canada, 2021). Market demands for meat in turn leads to a higher demand for livestock feed, including forages. With these increased requirements for good and services, agriculture is increasingly forced to sustainably intensify. Agriculture production can be increased by converting unused land to agricultural land, or intensifying productivity of existing agricultural land. Previously, when intensifying agriculture, producers have implemented the use of fertilizers, pesticides to increase crop productivity. Crop productivity also increased with better genotypes. However, there is an inherent limit to this approach, as improper genetic selection could lead to easier lodging (Guo et al. 2021), just as an excess of fertilizer can reduce crop yield (Liang et al. 2021; Qiang et al. 2020; Hendricks et al. 2019). Nitrogen availability is one of the most limiting factors affecting yield and quality of annual crop forage production systems. There is a growing concern with leaching as a main source of loss in the nitrogen cycle where many studies urge producers to procure new management practices to mitigate the poor nitrogen utilization (Bowles et al. 2018, Leach et al. 2004).

Recent studies have re-examined concepts such as the “Three Sisters” cropping method to increase productivity (Eames-Sheavly 1993). Maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), and squash (*Cucurbita spp.*) were the crop species typically integrated in the “Three Sisters” system by the Native Americans. The idea behind this cropping method is that when contrasting plant species were grown together, they can grow better than when these plants species grow separately. In modern agriculture, this practice is known as intercropping, and recent studies have identified many factors that influence intercropping yield (Du et al. 2016, Zhang et al. 2008, Yang et al. 2018). This would include the crop species selected, type of intercropping (alternate, mixed, 1:2 row arrangement), and harvest method (altogether or by crop maturity). Because of its overyielding effect (Xiao et al. 2019), intercropping is considered a sustainable agricultural practice as the overyielding effect could help reduce the amount of fertilizer used, which would then decrease the overall input needed for a season. One of the benefits of intercropping is efficient resource use through niche differentiation and complementarity (Willey 1985). Intercropping involving legumes not only can result in high yield but also can promote the uptake of nitrogen by crops (Brooker et al. 2016). Environmental conditions and agronomic management practices, such as N fertilization can affect crop production within intercropping systems. Inadequate soil N availability can affect crop growth and production. Nitrogen can also improve biomass and subsequently increase nutrient uptake and enhance nutrient balance and biomass production (Egan et al., 2019; Diaz et al., 2006).

In modern agricultural production, there are many tools to estimate crop productivity. One common method that is often applied to conventional agricultural systems is the normalized difference vegetation index (NDVI). Previous studies have shown that NDVI can be correlated to intercropping yield (Diatta et al. 2020, Sharaiha and Ziadat 2008), thus making it an extremely useful tool to image and identify improvements, if any, when optimizing inputs for intercropping. Alternatively, recent studies have examined the use fluorometry to estimate plant stress (Buchner et al. 2017, Perez-Molina et al. 2020). These studies demonstrated that under stress conditions such as drought and nitrogen deficiency, photosynthetic activity is reduced, thus a fluoremeter sensor can be used as a proxy measurement of plant stress instead of using visual scouting or foliar composition tests.

Intercropping is a practice that has garnered interest among producers in the Peace region located in northwestern Alberta, Canada. However, the existing literature does not represent the effects of intercropping under the unique cold climate, short growing season and dominant soil conditions of the Peace region. This study examines the benefits of oats (O, *Avena sativa* L.), peas (P, *Pisum sativum* L.), and canola (C, *Brassica napus* L.) intercropping systems on forage yield and nutritional quality focusing on livestock feed, specifically beef cattle diets. Furthermore, this study explores the underlying relationships between forage biomass yield and potential productivity indicators such as NDVI, NU_tE, and fluorometer readings.

2.3 Materials and Methods

2.3.1 Site Description and Experimental Design

Field experiments were carried out over two growing seasons from 30 May to 30 August 2019, and from 22 June to 21 Sept 2020, at the Fairview Research Farm, located in north-western Alberta, Canada (Lat: 56°04'53.3"N, Long: 118°26'25.1"W; 670 m above sea level). The study was conducted using new experimental plots each year, which were less than 100 m apart. This region is prone to long and cold winters and short and mild summers, hence characterized as a boreal climate. The soil at the experimental site is an Eluviated Black Chernozem, according to the Agricultural Region of Alberta Soil Inventory Database (AGRASID; GOA 2020). Prior to the commencement of the study in 2019, the management history of the experimental sites was a long-term (>10 years) alfalfa stand for hay production until termination with Roundup WeatherMax® herbicide (glyphosate) in the fall of 2018 and later deep plowed. In the spring of 2019, the site was disced and harrowed twice before seeding in 2019. The area used in 2020 for the study was left to fallow (chemical fallow) in 2019. The 2020 site was harrowed before seeding.

Weather data during the two growing seasons as well as the long-term averages were acquired through the Alberta Climate Information System (ACIS 2020) weather station located on site (Table 1). Baseline soil sample collection and analyses were done before crop seeding in both years. Soil availabilities of nitrogen (N) and phosphorus (P) were deficient (Table 1).

The three crop species chosen for this experiment were oats (O), peas (P), and canola (C), and were intercropped in all possible double-crop combinations producing the oat-pea (OP), pea-canola (PC), oat-canola (OC), and also the triple oat-pea-canola (OPC) intercropping. For monocrop oats [cv. CDC Seabiscuit (2019), CS Camden (2020)], peas (cv. CDC Meadow) and canola (cv. CS2500CL), the plant density goal is 300 plants m^{-2} (156 kg ha^{-1} in 2019, 154 kg ha^{-1} in 2020), 90 plants m^{-2} (246 kg ha^{-1} in 2019, 238 kg ha^{-1} in 2020), and 76 plants m^{-2} (3.91 kg ha^{-1} in 2019, 3.50 kg ha^{-1} in 2020), respectively. These seeding rates were corrected for thousand seed weight differences between the study years). Within 2-species intercroppings, each plant species was seeded at 75% of monoculture normal seeding rate, and in 3-species intercropping, each plant species was seeded at 50% of monoculture normal seeding rate. The method of intercropping chosen for this study was mixed intercropping. A factorial design in randomized complete blocks with four replicated was used. The two factors were intercropping systems, which consisted of the seven monocultures and intercropping options (O, P, C, OP, PC, OC, OPC) and four nitrogen (N) fertilizer (granular urea, 46-0-0) rates [0 (0N), 50 (50N), 75 (75N), and 100 (100N) kg N ha^{-1}]. We used a 6-row custom seeder (Fabro Enterprises Ltd., Swift Current, Saskatchewan, Canada) equipped with disc-type openers with a 22.9 cm row spacing to seed into plots that were 2 m wide and 16 m in length. Seeding was done on 30 May 2019 and 22 June 2020. A uniform P, K, and S fertilizers were applied to all plots at seeding (Table 1). The N fertilizers rates were also applied at seeding.

2.3.2 Crops management

To reduce weed competition, pre-emergent (2019) and pre-pass (2020) herbicide applications were carried out using StartUp® Roundup (glyphosate) herbicide at a rate of 1.66 L ha^{-1} and hand weeding was occasionally carried out in both years, where necessary.

2.3.3 Forage dry matter yield assessment

For forage yield determination, C was harvested at canola mid-podding stage (14 Aug. 2019; 31 Aug. 2020), O and OC was harvested at oat late-milk stage (21 Aug. 2019; Sept 1. 2020), and P, PC, OP, and OPC was harvested at first pod wrinkles observed in peas (22 Aug. 2019; 1 Sept. 2020). The aboveground parts of plants were hand harvested in 2019, while in 2020, a custom-made forage harvester [Swift Machine and Welding Ltd. (Swift Current, Saskatchewan, Canada) custom forage harvester, 63.5 cm wide] was used for harvesting. Three inner rows (at least 2 m in length) were harvested every year. The outer rows were not harvested to avoid edge effects. The crops were cut leaving an approximate 10 cm stubble. The harvested plant materials were weighed fresh. A biomass subsample of approximately 700 g per plot was dried to constant weight to determine the dry matter content, and dry matter yield was calculated.

2.3.3 Normalized difference vegetation index and photosynthetic rates

The canopy normalized difference vegetation index (NDVI) (GreenSeeker Handheld crop sensor, Trimble Ag, California, USA) and the photosynthetic efficiency of photosystem II (Y[II]) was measured using specialized tools (MINI-PAM II fluorometer, WALZ, Germany) at early flowering stage.

NDVI was measured in both years by positioning the sensor 31 cm above the canopy cover and in the centre of the plots and passing through the plots twice to obtain a plot average for NDVI.

Photosynthetic activity Y[II] was measured only in the growing season 2020, where one plant of each species present in the plot was selected. The newest mature leaf of each plant was dark-adapted with a specialized leaf clip provided with the fluorometer, which is then used to determine the minimum (F0) and maximum (Fm) photosynthetic activity for estimating Y[II].

2.3.4 Forage nutritive value analysis

With the aim of evaluating forage nutritive quality at harvest, forage samples were collected and sent to a commercial laboratory (A&L Canada Laboratories, London, Ontario, Canada), where standard procedures for wet chemistry and a near infrared protocol were used. Dried samples were ground with a Wiley mill to pass a 1-mm screen and analyzed for crude protein (CP) content. The samples were analyzed using Dumas direct combustion method and LECO FP628 nitrogen analyser to determine total N content, after which CP was calculated as %N x 6.25 (AOAC 1984). Forage minerals (macro- P, K, Ca, Mg, S, Na; micro- Zn, Fe, Mn, Cu) were determined by wet chemistry, using EPA Method 6010D (SW-846): Inductively Coupled Plasma - Atomic Emission Spectrometry (U.S. EPA, 2014) and Western States Laboratory Proficiency Testing Program Soil and Plants Methods (Miller et al., 1998). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using the ANKOM methods 5 and 6, respectively (ANKOM 200 Fiber Analyzer, ANKOM Technology, Macedon, NY). The NDF digestibility (NDFD) was determined from in vitro true digestibility in Ankom DaisyII incubator (Ankom Technology® method 3, Macedon, NY, USA), as described by Ammar et al. (1999). The NDF disappearance rate was based on the method provided by Doane et al. (1997). Net energies for maintenance (NEm), lactation (NEl) and gain (NEg), and total digestible nutrients (TDN) were calculated according to the equations provided by Adams (1980). Relative feed value (RFV) was also calculated as per Undersander et al. (2002). Nutritive value parameters are reported on a DM basis.

2.3.5 Calculation of crude protein yield and N utilization efficiency

Crude protein (CP) yield per hectare was calculated by multiplying crop forage yield (Mg ha^{-1}) by CP content to allow a comparison of CP yield potential of the different treatment combinations (intercropping × N rates) for animal feed production. N utilization efficiency (NU_tE) for biomass accumulation was calculated according to the following formula $NU_tE = DM/N$ (López-Bellido and López-Bellido, 2001), where DM is the dry matter at harvest and N is the total N uptake by the crop.

2.3.6 Statistical Analyses

Data collected was processed on a yearly basis through the R statistical program (R Core Team 2020), using the NLME program (Pinheiro et al. 2020) to complete a two-factor ANOVA analysis including crop biomass, forage nutritive measurements, NDVI and the photosynthetic efficiency of photosystem. A Tukey HSD post-hoc test using the base program ANOVA code (R Core Team 2020) and the post hoc test from the Agricolae package (de Mendiburu 2020). When significant interactions were found between N fertilizer addition and intercropping systems, we used ggplot2 to produce box plots (Wickham 2016).

2.4 Results

2.4.1 Forage Dry Matter Yield

There was a significant interaction between N fertilizer rates and intercropping systems when evaluating biomass production in both years of the study ($P < 0.05$, Table 2). For each intercropping treatment, there was a noticeable threshold for the optimal amount of N fertilizer for best yield. For example, PC at 75N provided the highest yield, while yield decreases occurred with the other N fertilizer addition rates (50N, 100N) in both years (Fig. 1). However, in 2019, PC at 0N did yield more than PC at 75N (Fig. 1A). The highest yielding intercropping system was OC at 0N, with 11.6 Mg ha^{-1} in 2019 (Fig. 1A). With OC, additional N fertilizer produced a decrease in yield (Fig. 1A), while OP still saw an increase in yield at 100N. P and C have relatively similar yields up until 100N, where P saw an increase in yield and C saw a decrease in yield (Fig. 1A). In 2020, O system at 0N yielded the highest biomass, resulting in 9.10 Mg ha^{-1} (Fig. 1B). OP at 100N in 2020 yielded 8.90 Mg ha^{-1}, which is similar to O at 0N in 2020. Monocrop systems seem to show a decreasing biomass yield with increasing N fertilizer rate in 2020 (Fig. 1B). Conversely, intercropping systems seem to perform in general better with increasing fertilizer additions in 2020 (Fig. 1B). In both 2019 and 2020, OPC maintained relatively similar yield, despite the different climate conditions between the two study years, and the different rate of N fertilizer addition (Fig. 1).

2.4.2 Forage Nutritional Content

In both years, none of the forage nutritive indicators determined in this study had significant intercropping and N rate interaction effects (2019, P value > 0.11; 2020, P value > 0.38) (data not shown). Except for NDF Disappearance Rate, which was not affected by intercropping treatments in 2020, other forage nutritive value measurements were impacted by intercropping treatments in both years. In both years, forage nutritive parameters measured in the present study were mostly not affected by N fertilizer rates (data not shown).

2.4.2.1 Intercropping Systems Effect

In both years, there were significant differences in CP between intercropping options (2020, $P < 0.01$; 2019, $P < 0.01$, Table 3). In 2019, C and P systems performed best in CP, where C resulted in 17.1% and P had 15.7% (Table 3). PC intercropping resulted in a CP content of 13.7%; this result was similar to OC, but was significantly lower than P and C. The cropping treatments that contained oat alone or as part of intercropping, had similar CP content and mostly in the bottom (Table 3). In 2020, results of CP were the highest in three systems: C alone, P alone, and the PC combination, where CP contents were 16.2%, 15.1%, and 15.4%, respectively. Although CP content in OP (14.5%) did not statistically differ from P and PC, CP in OP was more similar to that of the other cropping systems that contain oats. O, OPC and OC intercropping resulted in significantly less CP than the other cropping options.

In 2019, P had the highest TDN content, at 67.6% ($P< 0.01$, Table 3). O, C, OP, and OC had TDN contents between 65.0% and 66.0%. PC (63.9%) and OPC (64.1%) had lower TDN content than others. In 2020, C produced a TDN content of 62.4%, whereas the other intercropping systems produced higher TDN between 65.0% and 67.0%.

The forage ADF and NDF contents for both years are shown in Table 3. In 2019, PC, OPC, and O produced significantly higher ADF (36.4-38.3%) than P, which produced the lowest ADF value with 32.7%. However, P had some form of similarity ($P > 0.05$) in ADF content to C, OP, and OC. In contrast, C alone in 2020 (37.4) had greater ADF than P, O, OP, and OC at 35.3%, 34.4%, 35.3%, and 35.0%, respectively. PC and OPC were indifferent to other forage production systems. In 2019, intercropping options that included oats alone or in combination were similar to each other; however, they had significantly higher NDF than pea and canola combined (P, C, and PC). Options including oat (i.e., O, OC, OPC, OP) yielded NDF between 51.0% and 56.5%, while the options including pea and canola resulted in NDF between 42.0% and 46.0%. In 2020, OP (48.5%) greatly outperforms PC (45.9%) and C (44.5%) in terms of NDF.

P was highly different than both OPC and PC in all three net energy indicators (NEL, NEM, NEG), and the rest of the forage production systems (P, OPC, and PC) had similar net energy results (Table 3).

In 2019, P has the highest RFV with 147 ($P<0.01$). Besides C, P was significantly higher than the other intercropping systems, which have RFV between 101 and 122 (Table 3). OP (112), PC (122), and OPC (108) had slightly higher RFV than OC (105) and O (101).

In 2020, where NDFD at 24- and 48-hr, and NDF disappearance rate were examined, O consistently had the highest values in NDFD-24hr and NDFD-48hr (Table 3). While C and PC had similar values for NDFD-24hr, PC was marginally higher than C in NDFD-48hr.

Concentrations of minerals in forage varied with intercropping choice and study year (Table 4). In 2019, P and PC had higher calcium content (1.42 - 1.45%) than others. Furthermore, C was significantly higher than OP, but not significantly higher than OPC. OP significantly had the lowest calcium content with 1.01%. Overall, O and OC had the lowest calcium content, with 0.41% and 0.44%, respectively. In 2020, C produced the highest calcium content at 1.68% and differed significantly from others. PC and P had the second highest calcium content with 1.38%

and 1.37%, respectively. Forage production systems containing oats (i.e., OPC, OC, O, OP) produced the lowest calcium concentrations.

C had the significant highest phosphorus content in 2019 (0.375%). In 2020, C also had the highest phosphorus content with 0.311%, and OC had the lowest (0.180%). Although P and PC were lower than C, they contained somewhat significantly more phosphorus content than the four oat-based systems.

Potassium content was the highest in C, with 2.24% in 2019 and 2.01% in 2020. In 2019, OPC and OP produced the lowest potassium content in plant tissues, yielding 1.49% and 1.48%, respectively. Similarly, OPC and OP also had the lowest potassium concentrations in 2020. In 2020, P produced the second highest concentration (1.32%), and OC produced the lowest potassium content (0.968%). However, within 2019, OC had a potassium content similar to C, and P showed one of the lowest performances.

While the ANOVA test indicated significant differences for magnesium in 2020, the post-hoc test was unable to detect significant differences between the intercropping systems, due to the high coefficient of variation (CV). In 2019, PC produced the highest magnesium content (0.486%). OP and P produced significantly lower magnesium contents, with 0.409% and 0.408%, respectively. OPC (0.434%) was not significantly different than either PC or OP. O, C, and OC showed the lowest contents across all assessed intercropping systems.

In 2019, O, OC, and C resulted in significantly higher sodium content than the rest of the intercropping systems, resulting in 0.100%, 0.100%, and 0.091%, respectively.

2.4.2.2 N fertilizer rates effect

In 2019, the only forage nutritional parameters that were influenced significantly by N fertilizer rates were TDN and RFV. However, both TDN and RFV were only marginally impacted by N fertilizer rates (ANOVA P= 0.05). TDN had a narrow range from 65.8% in the 0N control to 69.2% with the highest N rate (100N) (data not shown).

2.4.3 Crude protein yield and utilization efficiency

CP yield, calculated as the product of biomass and CP content, showed a significant interaction between intercropping systems and N fertilizer rates in 2020, but not in 2019 ($P < 0.01$, Table 2); however, intercropping systems (pooled across N fertilizer rates) showed clear significant impacts on the overall CP yield ($P<0.01$) in 2019, but not N fertilizer rates, when averaged across intercropping systems. In 2019, the CP yield in OPC (937 kg CP ha^{-1}) was significantly the lowest intercropping system (data not shown), while OC was the highest (1174 kg CP ha^{-1}). The other intercropping systems were very similar, ranging between 1022-1117 kg CP ha^{-1}. In 2020, P at rates of 50N, 75N, 100N showed the least CP yields, while OP at 100N had the highest CP productivity (Fig. 2).

In 2019 and in 2020, we observed no interactions between factors, and only intercropping systems affected NU_tE ($p < 0.01$, Table 2). Within both growing seasons, no significant interactions between N fertilizer addition and intercropping systems were observed (data not shown). The highest NU_tE was derived from OP (56.6), OPC (55.9), and O (55.5) in 2019, while the lowest was P (40.0) and C (36.9). OC (51.7) was relatively high as well in 2019 (Fig. 3A). However, in 2020, OC (45.9) had the highest NU_tE, along with O (45.8) and OPC (45.6) (Fig. 3B). While P (41.5) is comparable to PC (40.7) in 2020, C (38.6) remains the poorest crop for NU_tE.

2.4.4 NDVI and Photosystem II

There was no significant interaction effect of intercropping x N fertilizer rates on NDVI in 2019, but in 2020, we did observe significant interaction between intercropping x N rates. In 2019, we saw significant NDVI differences between intercropping systems ($P < 0.01$). C had the poorest NDVI while O had the greatest, at 0.60 and 0.83, respectively. OC and OPC performed relatively similar, producing an NDVI of 0.76 and 0.75, respectively. OP performed slightly better than OC and OPC with an NDVI of 0.81. PC had an NDVI of 0.67, which was slightly below P (0.70). In 2020, O at 0N was significantly higher than all other treatments, including O at different N fertilizer rates (Fig. 4). P and PC saw a positive influence on NDVI with increasing N fertilizer (Fig. 4). Contrasting P and PC, OC saw a negative influence on NDVI with additional N fertilizer. OP and OPC remained relatively constant in terms of NDVI (Fig. 4). C at 50N could be the threshold for N fertilizer applications as NDVI decreased with N fertilizer rates beyond 50 kg N ha^{-1}, and that NDVI increased when comparing 50N versus 0N.

The following regressions of forage yield quantity and quality against NDVI provided some insights into how NDVI relates with yield quantity and quality parameters:

$$\begin{aligned}\mathrm{NDVI2019} = {} & 1.38({*}{*}{*}^{\dagger}) - 6.95e^{-3} Moisture({*}{*}) - 9.98e^{-3} CP({*}{*}) \\ & + 3.09e^{-3} TDN({*}{*}) - 4.27e^{-1} Phosphorus({*}{*}) \\ & + 3.52e^{-2} Potassium - 1.56e^{-1} Magnesium({*}) \\ & - 4.16e^{-1} Sodium({*}) - 7.53e^{-6} Biomass\ Yield\end{aligned} \tag{1}$$

$$
\begin{aligned}
\text{NDVI2020} = {} & 7.71e^{-1}(***) + 7.59e^{-2}Moisture - 5.11e^{-3}CP - 1.00e^{-4}TDN \\
& - 9.17e^{-2}Calcium(**) - 1.36e^{-2}Potassium \qquad [2] \\
& - 7.47e^{-2}Magnesium + 1.46Sodium + 5.30e^{-6}Biomass\ Yield(*)
\end{aligned}
$$

†: The asterisks in the regression terms indicates significance.

* is $P < 0.05$, ** is $P < 0.01$, *** is $P < 0.001$

where Eq. [1] for 2019 accounted for 61% of the changes in NDVI ($P <0.01$) and Eq. [2] for 2020 accounted for 43% of the changes in NDVI ($P <0.01$).

There were no significant interactions and differences between treatments N fertilizer additions in oats ($P > 0.05$), peas ($P > 0.05$), and canola ($P > 0.05$) when measuring Y[II] (data not shown).

2.5 Discussion

2.5.1 Forage Production, Canopy Cover NDVI and N utilization Efficiency

In our study, competitive monocrop systems (oat and canola) generally yielded the highest in terms of biomass yield. This is consistent with Kontturi et al. (2011), Neugschwandtner and Kaul (2015), and Bacchi et al. (2021), where the introduction of an intercrop significantly lowered overall yield compared to monocrops, regardless of ratios or seeding rates. While the seeding ratio in Bacchi et al. (2021) was 50:50, in the present study, we overseeded oat-pea intercropping at 150% of the total seeding rate while maintaining 75:75. Kontturi et al. (2011) also found a significant advantage of monocrop oat over intercrops in wet years, despite having ratios of pea:oat such as 7.5:92.5 and 15:85. Relative to these studies, Neugschwandtner and Kaul (2015) was the only study that observed peas as the highest yielding monocrop, but the overall trend of intercropping yielding less than monocropping consistently remains.

In 2020, where the highest yielding crop was O at 0N, we suspect that N fertilizer additions negatively impacted oat growth. In the soil at the study sites (i.e., Eluviated Black Chernozem), the recommended N fertilizer rate does not exceed 44.8 kg N ha^{-1} (GOA 2004), whereas the N present in the soil prior to seeding far exceeds these values (Table 2), thus causing a decrease in yield with N fertilizer addition (Liang et al. 2021).

We observed certain cases where a highly N fertilized (100 kg N ha^{-1}) oat-pea intercropping system in a wetter year (2020) had biomass productivity like monocrop oats, and an oat-canola intercropping system with no fertilizer addition yielded higher than monocrop oats in a drier year (2019). Earlier reports indicate that oat-pea intercropping could outperform monocropping under specific environments that matched our study (Pflueger et al. 2020, Han et al. 2012). As noted by Han et al (2012), oat-pea intercropping was able to over-yield in the presence of high N fertilizer addition. The same was observed in our study as OP at 100N was able to outperform all monocropping treatments specifically in 2020, but not in 2019. This interannual variation could be explained by the high variability that differences in moisture content creates for the oat-pea system (Pflueger et al. 2020). With higher soil moisture content, oat-pea systems have increased probability to yield higher than monocrop systems. However, relative to oat-pea intercropping, the oat-canola system remains understudied. While productivity was increased with increasing N application rate in drier years for wheat-canola intercropping in an experiment by Ebrahimi et al (2016), the same was not observed for oat-canola intercropping. In fact, the opposite was observed, where the addition of N fertilizer decreased yield. Likewise, Shoaib et al. (2014) was not able to produce a yield that outperforms monocrop oats on average. This could potentially mean that oat-canola outperforming oats in our study could be the effect of the specific weather and soil conditions in our study, and this intercropping combination requires further investigations.

In terms of nitrogen utilization efficiency (NU_tE), we wanted to understand the efficiency of converting access to N into dry matter yield. Contrary to Neugschwandtner and Kaul (2015), oat alone did not produce the highest NU_tE, nor did fertilization affect the NU_tE values. In fact, intercropping oats (OC, OP, OPC) produced similar, if not higher, NU_tE as oat alone. That said, compared to Neugschwandtner and Kaul (2015), this present study did not observe grain yields, thus not demonstrating the effects of N fertilization comprehensively. With pea and canola intercropping, in a drier year, the plants could be not competing as the taproot might not be interacting (Lee et al., 2021), but as the taproot size increases, competition for resources becomes apparent as pea alone was able to produce higher NU_tE than PC in 2020. With limited availability of studies focusing on pea and canola, more research should be dedicated to understanding why this occurs.

Normalized difference vegetation index (NDVI) is a practical indicator of field productivity (Diatta et al. 2020), but with competitive plants such as oats, NDVI may be biased towards the denser fields. However, by using this metric, we can determine which crop is best suited for intercropping in oats. In this study, we found that OP had the best NDVI compared to other intercropping systems that contained oats (OC, OPC), which would lead to choosing OP as the ideal choice for intercropping comparatively. Furthermore, this ideal is further backed by the fact that OP was also capable of producing the most biomass yield compared to OC and OPC (Fig. 1). Similarly, PC showed that by intercropping peas and canola, we can reduce the volatility of pea yield. However, since the determining factor of NDVI is more than just biomass yield, this method of determining best practice requires supplementary indices.

As the regression analyses were able to explain about 50% of the changes in NDVI, we noticed that the reliability of the regression depends on the climate conditions. In the dryer year (2019, Eq. [1]), the regression indicates that the quality parameters of the crop is the significant factor influencing NDVI, while in the wetter year, the productivity of the crop is the significant factor influencing NDVI. This effect likely occurred because in a dry year, with limited access to mobile nutrients, the plant is required to spend extra energy to obtain nutrients, thus leading to the difference in quality. The efficiency of the plant in acquiring nutrients essentially determines the productivity of the plant (Htoon et al. 2014). Conversely, where nutrients are mobile due to moisture sufficient soils, there is an emphasis on passive uptake of nutrients through ion exchange (Simunek and Hopmans 2009). This could be a reason as to why the marginal differences in NDVI were smaller in the wetter 2020.

While there were no significant differences in Y[II] between oats, peas, nor canola under different plot treatments, we believe it is because we did not factor in leaf area. As it is possible to detect changes in Y[II] under stressed conditions such as drought stress, nitrogen deficiency, and heat stress (Buchner et al. 2017, Haque et al. 2014, Perez-Molina et al. 2020, Xu and Mou 2016), it would indicate that differences in Y[II] is not distinguishable until presented in extreme conditions. That said, while the plant's Y[II] is working optimally, to determine the plant efficiency, it would need to be paired with the leaf area (Leaf area index, LAI), as the sampled area using the fluorometer is a fixed size.

It is a well-known fact that legumes are plant species with high protein content, which comes to no surprise that in terms of crude protein concentration that pea would yield best (Table 3). Interestingly, pea-canola intercropping decreased the crude protein yield. Upon calculating the product of protein concentration by biomass productivity (Fig. 3), we found that the main driver of this protein productivity by PC was protein concentration. Pea-canola intercropping produced a higher biomass content and a lower crude protein content compared to pea or canola grown alone. This result may indicate that there is a limit to crude protein production and indicates that intercropping can be used as a method to manage trade off between biomass vs. crude protein production for optimal economic gain.

2.5.2 Forage Nutritive Value Indicators

Crude protein is one of the key objectives of livestock agriculture, specifically cattle management, strives for. In general, all forages meet and in most cases far exceed the CP requirements of mature beef cattle, which require 7% CP in mid-pregnancy, 9% in late-pregnancy and 11% CP for cows with calves (NASEM, 2016), therefore eliminating the need for protein supplementation with any of the forage production systems investigated in our study. Unlike in earlier reports (Ullah et al. 2018, Yilmaz et al. 2015, and Gill and Omokanye 2018), which showed that the crude protein of cereals could significantly be increased by the use of annual legumes in intercropping systems, the present study reported here does not realize a significant increase in forage CP from intercropping with oats over monoculture oats. However, far from Ullah et al. (2018), fertilizer application did not influence the CP yield in our study, and by adding either peas or canola into oat systems, the increase in protein content was actually negligible. One speculation that could be made is that increases in protein content emerge from the presence of legumes in the mixture (Gill and Omokanye 2018). This is consistent with the protein content remaining the same across cropping systems despite using two different varieties of oats in two different years (Seabiscuit in 2019 and Camden in 2020) in the present study.

In cold climate, energy is one of the important criteria for nutritive value evaluation, particularly in beef cattle production. The rule of thumb for a mature beef cow to maintain her body condition score through winter in the study area, 55-60-65%, explains that a ration must have TDN content of 55% for mid pregnancy, 60% for late pregnancy, and 65% for after calving (NASEM 2016; Yurchuck and Okine 2004). Using this rule of thumb, our study showed that intercropping systems can effectively meet the TDN requirements of different categories of beef cows, with a few exceptions. In 2019, C, PC, and OPC met only the requirements up to the late pregnancy period, while the rest of the intercropping options met the high requirements for after calving. In 2020, only C failed to meet the requirement for after calving. By intercropping pea or oat with canola (i.e., OC and PC), there is a general increase in TDN content from C alone, indicating either oat or pea in combination with canola could be used to improve canola forage TDN content. However, compared to O alone, there was a decrease in TDN when intercropped, even when using different varieties of peas (Gill and Omokanye 2018). However, this decrease was generally negligible as the forage mostly met the highest demand for TDN.

PC and OPC performed worse than their monocropped counterparts in 2019 in terms of net energy estimations, but they saw an improvement in net energy values compared to C in 2020 (Table 3). The results obtained in the two years of the study exceeds the NASEM (2016) recommended NEM and NEG values of 1.19-1.28 Mcal kg^{-1} and 0.53-1.37 Mcal kg^{-1}, respectively. This cautions the use of canola in intercropping in a nutrient poor soil on a dry year.

NDF and ADF are effective indicators of the digestibility of the feed (Vaezi Rad et al. 2020). Pea and canola monocrops generally produced the lowest NDF content, and by intercropping these two crop species, a slight increase was observed (Table 3). OP and OC produced an improvement in NDF, which is consistent with barley-pea intercropping in Javanmard, Machiani, and Eskandari (2019). In a wet season (2020), ADF fluctuations between systems were minimal, but intercropping produced similar ADF content to its monocropped plant, if not lower. In 2019, intercropping increased ADF content, as previously found by Omokanye (2014) and Vaezi Rad (2020). Specifically, the presence of pea and canola in intercropping systems increased the ADF content. While there was a noticeable increase in PC and OPC above the average ADF, the overall results were well within expected values for ADF.

Besides ADF and NDF, the other nutrition factor to consider is the NDF digestibility, which is measured as the dissolved NDF content in-vitro in this study (Hoffman et al. 2001). In 2020, we were able to acquire data on NDF digestibility in 24 hours and 48 hours. While most intercropping systems demonstrated that the digestibility is generally the median of the crop species in the system, there were some cases where the intercropping system had better digestibility than its monocropped counterpart, such as PC having a NDFD24 ~1% lower than P and C. Intercropping can change the microclimate and growing environment, thus would exhibit change in the NDF content (Hoffman et al. 2001), but how the crop growing environment changes is not well known. This study therefore recommends future research to examine whether plants are either aging slower or faster over the growing season in intercropping systems by testing the digestibility indicators (ADF, NDF, NDFD).

Based on NASEM (2016) and Omokanye (2014), most forage mineral contents achieved the necessary mineral requirements for beef cattle in our study as abovementioned. Calcium requirements (0.57%) were generally attained by all the systems, apart from O and OC in 2019. The addition of canola to oats seems to positively influence calcium content (Omokanye 2014), but the difference was not significant and would need more tests to confirm. OPC produced the most consistent calcium content throughout the two years. Phosphorus requirements for lactating cows (0.26%) were conveniently met in 2019 except canola and OP, which just fell short of 0.26% P. Sparingly, canola phosphorus content was higher than needed and was the only crop to meet the requirement in 2020. Canola is the most efficient phosphorus using plant species of the 3 crop species used in this study, and from our results, we witness that by adding other crop species to canola, we hampered the system's ability to effectively attain high phosphorus content. Rather, the addition of canola to the oat or pea system improves phosphorus content overall, which could be seen as an advantage when aiming at low phosphorus content in forage biomass. C was once again the crop with the highest potassium concentrations, and intercropping canola hampered this ability to accrue potassium. However, the present study consistently exceeds the potassium requirement (0.70%) of mature beef cattle but was just below the maximum allowable amount (3%) of potassium for pregnant and nursing cows (NASEM 2016, Omokanye 2014). By intercropping oats, peas, and canola, there was a significant reduction in potassium content, which is ideal in the scenario where feed is generally too high in potassium. The present study observes an unusually high magnesium content compared to previous studies, as well as an unusually low sodium content (Gill and Omokanye 2018, Omokanye 2014). In a drier year (2019), intercropping (OP, PC, OPC) favoured increases in magnesium uptake. The same effect was not observed in the wetter year (2020). Similarly, sodium was unaffected during the wetter year (2020), but observed a general decrease in sodium content in the drier year. OC, however, was able to produce the same concentration of sodium as O in the drier year. Overall, OPC provided steady mineral contents throughout both trial years, with minimal fluctuation.

As the relative feed value (RFV) demonstrates that all intercropping systems were relatively similar in 2020, it would mean that in terms of quality, they all meet the basic requirements of a cattle, which, as seen in the forage nutritive value indicators including digestibility and minerals, were exceeding the minimum requirements. Where moisture was less abundant, such as 2019 in this study, RFV varied between intercropping systems. It is therefore recommended that when choosing forage species for seeding in a drier year, one should consider which nutrient is more valuable to prioritize, in terms of economical cost and in terms of availability within the region, as most RFV results in this study are considered a “good” forage feed for cattle according to studies mentioned in Gill et al. (2013).

2.6 Conclusion

Intercropping oats, peas, and canola provides many different benefits, as well as caveats. In the present study, the overall trends comparing intercrops to their respective monocrops counterparts showed decrease for few intercropping options (OP, OC), improvement for some (PC, OPC) and no effect for others. Although certain patterns were not always significant, intercropping options did not reach in general the yield of oats alone. A few exceptions to this generalization can be highlighted. Of the tested systems, PC was the most valuable intercropping option because it matched the biomass productivity of both pea alone and canola alone and provided adequate nutritional quality for cattle feed. PC was also a very nitrogen efficient intercropping, as it was able to produce high biomass yield per unit of nitrogen present in the forage (NU_tE). Although OPC was low yielding, it was the system with most consistent nutritive value across the two years of the study. The mineral content in OPC forage was relatively similar between the two study years even though the weather conditions experienced across two years were very contrasting. Our results suggest that implementing PC intercropping can reduce external feeding inputs in livestock production because PC increased overall productivity, as well as increase mineral content, thus effectively reducing the cost of mineral supplements. This study indicates that one can balance diets for beef cattle easier when growing OPC intercropping because biomass and mineral yields become more consistent across production years. Additionally, this study indicates that canopy NDVI is a functional proxy of forage productivity and quality when laboratory tests are unavailable.

2.7 Acknowledgements

We would like to thank Liisa Jeffrey, Kaitlin McLachlan, Johanna Murray, Buthaina Al-Maqtari, Dong Hyeon Kim, Courtney Chaykowski, and Frances MacArthur for the continued support throughout the project. We would also like to thank Dr. Muyiwa Olayemi for providing guidance with statistical procedures in this study. This research was financially supported by the Canadian Agricultural Partnership (CAP) - Environmental Stewardship and Climate Change Group Program (ESP-048569).

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2.9 Tables and Figures

Table 2.1. Monthly rainfall and air temperature and their long-term averages during the two growing seasons of the experimentation, as well as the site soil characteristics and basal fertilizer applied.[1]

	2019	2020	Long Term Average
Month	Rainfall (mm)		
June	72.9	67.2	64.5
July	61.9	89.8	69.5
August	49.1	53.9	47.5
September	24.6	23.1	33.7
Total	208.5	234.0	215.2
	Temperature (°C)		
June	14.1	14.0	14.0
July	15.1	15.6	15.9
August	12.9	14.1	14.6
September	9.7	10.3	9.6
Soil property			
N (mg kg^{-1})	5	24	
P (mg kg^{-1})	7	10	
K (mg kg^{-1})	242	216	
S (mg kg^{-1})	5	5	
Ca (mg kg^{-1})	1920	1760	
Mg (mg kg^{-1})	354	359	
pH	6.2	6.2	
Organic matter, OM (%)	8.2	4.7	
Fertilizer Blend Applied			
P_2O_5 (kg ha^{-1})	27.2	10.9	
K_2O (kg ha^{-1})	27.2	Nil*	
S (kg ha^{-1})	12.7	13.6	

* Soil tests indicated adequate availability of soil K, so no additional K_2O was applied.

Table 2.2. ANOVA results of biomass yield, crude protein (CP) yield, and nitrogen utilization efficiency (NU_tE)[2]

	Factor	Df	P-2019	P-2020
Biomass Yield	Intercropping	6	<0.01	<0.01
	Fertilizer[a]	3	0.01	0.05
	Inter x Fert[b]	18	0.01	<0.01
CP Yield	Intercropping	6	<0.01	<0.01
	Fertilizer	3	0.21	0.39
	Inter x Fert	18	0.09	<0.01
NU_tE	Intercropping	6	<0.01	<0.01
	Fertilizer	3	0.09	0.09
	Inter x Fert	18	0.61	0.69

[a] N fertilizer addition

[b] interactions between intercropping systems and N fertilizer addition

Table 2.3. Tukey post-hoc of the ANOVA of forage quality indicators (dry matter basis) in 2019 and 2020[3]

	CP (%)	ADF (%)	NDF (%)	TDN (%)	NDFD 24* (% of NDF)	NDFD 48* (% of NDF)	NDF Diss Rate* (% hr^{-1})	NEL (MCal Kg^{-1})	NEM (MCal Kg^{-1})	NEG (MCal Kg^{-1})	RFV
2019											
O	11.4 c[+]	36.3 a	56.2 a	65.2 ab	-	-	-	1.37 ab	1.47 ab	0.75 ab	101 c
P	15.7 a	32.7 b	42.6 b	67.6 a	-	-	-	1.43 a	1.55 a	0.82 a	147 a
C	17.1 a	35.7 ab	44.7 b	65.6 ab	-	-	-	1.38 ab	1.48 ab	0.76 ab	128 ab
OP	11.2 c	35.5 ab	51.3 a	65.8 ab	-	-	-	1.39 ab	1.49 ab	0.77 ab	112 bc
PC	13.6 b	38.4 a	45.8 b	63.9 b	-	-	-	1.31 b	1.40 b	0.68 b	122 bc
OC	12.3 bc	35.2 ab	54.6 a	65.9 ab	-	-	-	1.39 ab	1.50 ab	0.77 ab	105 c
OPC	11.2 c	38.0 a	51.4 a	64.1 b	-	-	-	1.34 b	1.43 b	0.71 b	108 bc
P I	<0.01	<0.01	<0.01	<0.01	-	-	-	<0.01	<0.01	<0.01	<0.01
P F	0.08	0.08	0.17	0.08	-	-	-	0.11	0.11	0.11	0.05
P IxF	0.56	0.11	0.89	0.11	-	-	-	0.42	0.42	0.38	0.18
CV, %	12.2	5.44	9.57	3.65	-	-	-	5.57	6.10	12.0	17.2
2020											
O	13.8 c	34.4 b	47.4 ab	67.4 a	42.6 a	58.8 a	4.14 a	1.41 a	1.51 a	0.79 a	122 a
P	15.1 ab	35.3 b	46.3 abc	65.8 a	40.6 ab	54.8 ab	4.03 a	1.39 a	1.49 a	0.77 a	124 a
C	16.2 a	37.4 a	44.4 c	62.4 b	39.3 b	48.1 c	3.96 a	1.35 b	1.45 b	0.72 b	126 a
OP	14.5 bc	35.3 b	48.5 a	66.2 a	41.2 ab	57.2 ab	4.04 a	1.39 a	1.49 a	0.77 a	118 a
PC	15.4 ab	36.2 ab	45.9 bc	65.1 a	39.0 b	52.5 bc	4.04 a	1.37 ab	1.47 ab	0.75 ab	123 a
OC	13.7 c	35.0 b	46.5 abc	66.8 a	41.0 ab	56.5 ab	4.11 a	1.40 a	1.50 a	0.78 a	123 a
OPC	13.8 c	35.6 ab	47.9 ab	66.4 a	41.9 ab	57.2 ab	4.00 a	1.38 ab	1.49 ab	0.77 ab	119 a
P I	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.65	<0.01	<0.01	<0.01	0.07
P F	0.13	0.76	0.14	0.74	0.68	0.72	0.10	0.76	0.73	0.74	0.17
P IxF	0.69	0.8	0.47	0.72	0.38	0.59	0.66	0.80	0.84	0.82	0.68
CV, %	7.91	4.97	5.05	3.62	7.46	8.05	21.1	2.49	2.70	5.26	6.62

*, Not determined in 2019, so no data is available for 2019 growing season.

[+]: Factors with same letters within a column indicate no significant differences based on Tukey HSD test

CP: Crude Protein

ADF: Acid detergent fibre

NDF Neutral detergent fibre

TDN: Total Digestible nutrients

NDFD 24: NDF digested at 24 hours

NDFD 48: NDF digested at 48 hours

NEL: Net energy for lactation

NEM: Net energy for maintenance

NEG: Net energy for gain

RFV: Relative feed value

P I: Intercropping systems p-value

P F: N fertilizer addition p-value

P IxF: p-value of the interaction between intercropping systems and N fertilizer addition

CV, %: Coefficient of variation

Table 2.4. Means and Tukey post-hoc of the ANOVA of average forage mineral content (% dry matter basis) in 2019 and 2020.[4]

	Calcium, %	Phosphorus, %	Potassium, %	Magnesium, %	Sodium, %
2019					
O	0.41 d*	0.26 b	1.86 bc	0.19 d	0.10 a
P	1.45 a	0.25 b	1.69 cd	0.41 b	0.04 b
C	1.24 b	0.38 a	2.24 a	0.33 c	0.09 a
OP	1.01 c	0.25 b	1.48 d	0.41 b	0.03 b
PC	1.42 a	0.26 b	1.63 d	0.49 a	0.04 b
OC	0.44 d	0.27 b	2.08 ab	0.21 d	0.10 a
OPC	1.10 bc	0.26 b	1.49 d	0.43 ab	0.04 b
P I	<0.01	<0.01	<0.01	<0.01	<0.01
P F	0.28	0.27	0.41	0.46	0.48
P IxF	0.33	0.88	0.42	0.98	0.96
CV, %	14.2	12.7	17.8	15.9	36.0
2020					
O	1.09 c	0.21 bc	1.21 bc	0.36 a	0.03 a
P	1.37 b	0.23 b	1.32 b	0.41 a	0.03 a
C	1.68 a	0.31 a	2.01 a	0.40 a	0.03 a
OP	1.09 c	0.21 bc	1.22 bc	0.36 a	0.03 a
PC	1.38 b	0.22 b	1.18 bc	0.42 a	0.04 a
OC	1.14 c	0.18 c	0.97c	0.38 a	0.03 a
OPC	1.16 c	0.21 bc	1.25 bc	0.38 a	0.03 a
P I	<0.01	<0.01	<0.01	<0.01	0.02
P F	0.33	0.99	0.92	0.92	0.50
P IxF	0.77	0.70	0.58	0.68	0.54
CV, %	11.6	14.4	20.4	20.9	15.0

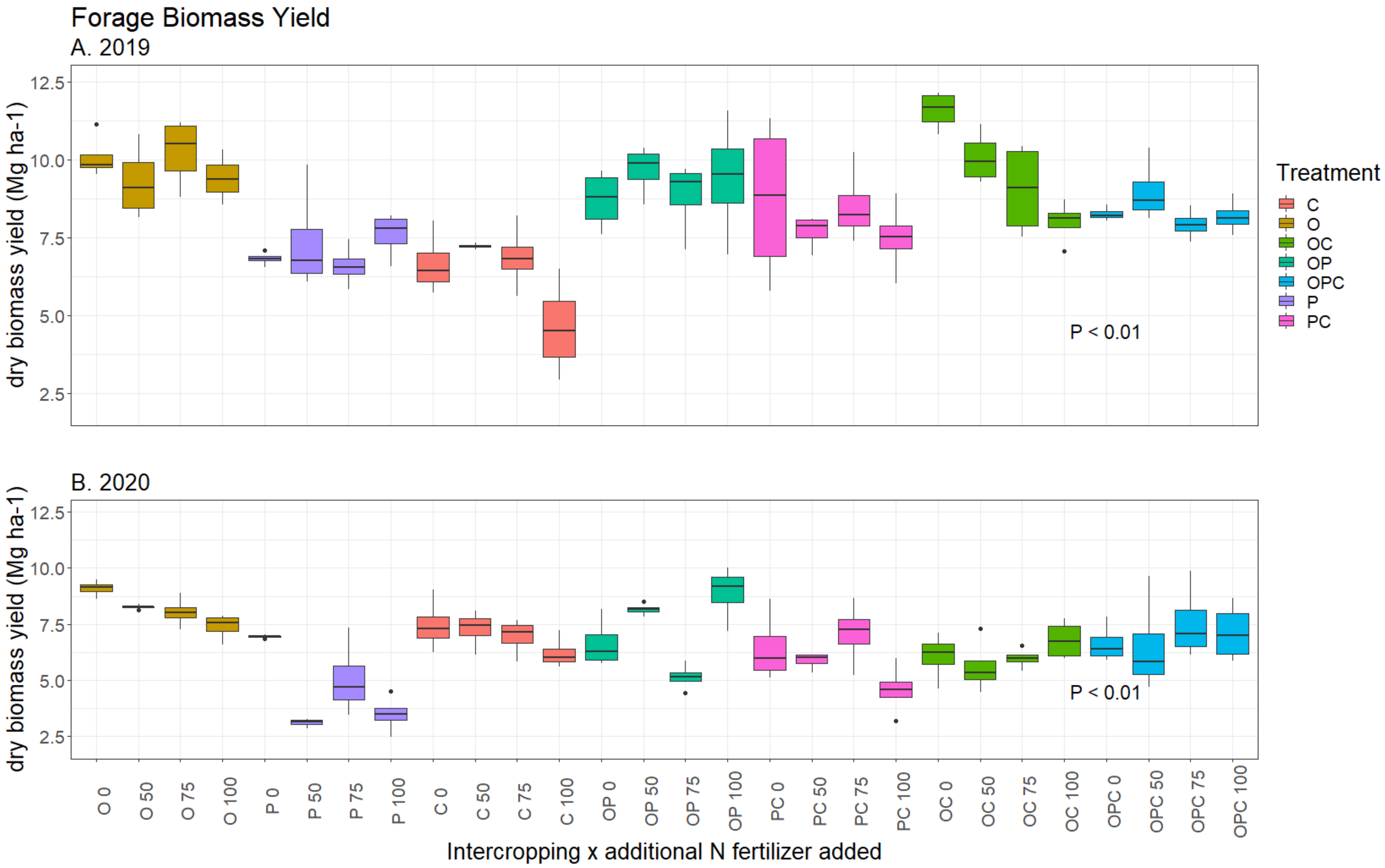
P I: Intercropping systems p-value

P F: N fertilizer addition p-value

P IxF: p-value of the interaction between intercropping systems and N fertilizer addition

CV, %: Coefficient of variation

***:** Factors with same letters within a column indicate no significant differences based on Tukey HSD test



Forage Biomass Yield
A. 2019
B. 2020
dry biomass yield (Mg ha-1)
12.5
10.0
7.5
5.0
2.5
P < 0.01
Treatment
C
O
OC
OP
OPC
P
PC
O 0
O 50
O 75
O 100
P 0
P 50
P 75
P 100
C 0
C 50
C 75
C 100
OP 0
OP 50
OP 75
OP 100
PC 0
PC 50
PC 75
PC 100
OC 0
OC 50
OC 75
OC 100
OPC 0
OPC 50
OPC 75
OPC 100
Intercropping x additional N fertilizer added

Fig. 2.1. Forage biomass yield (aboveground dry matter) collected in 2019 (A) and 2020 (B). In 2019 (A), OC 0N had the highest yield at 11.6 Mg ha^{-1}. In 2020 (B), O 0N (9.10 Mg ha^{-1}) and OP 100N (8.90 Mg ha^{-1}) had the highest yield. OPC was least affected by the climate differences between 2019 and 2020, as the yield difference between the two years was the smallest of the intercropping systems.[1]

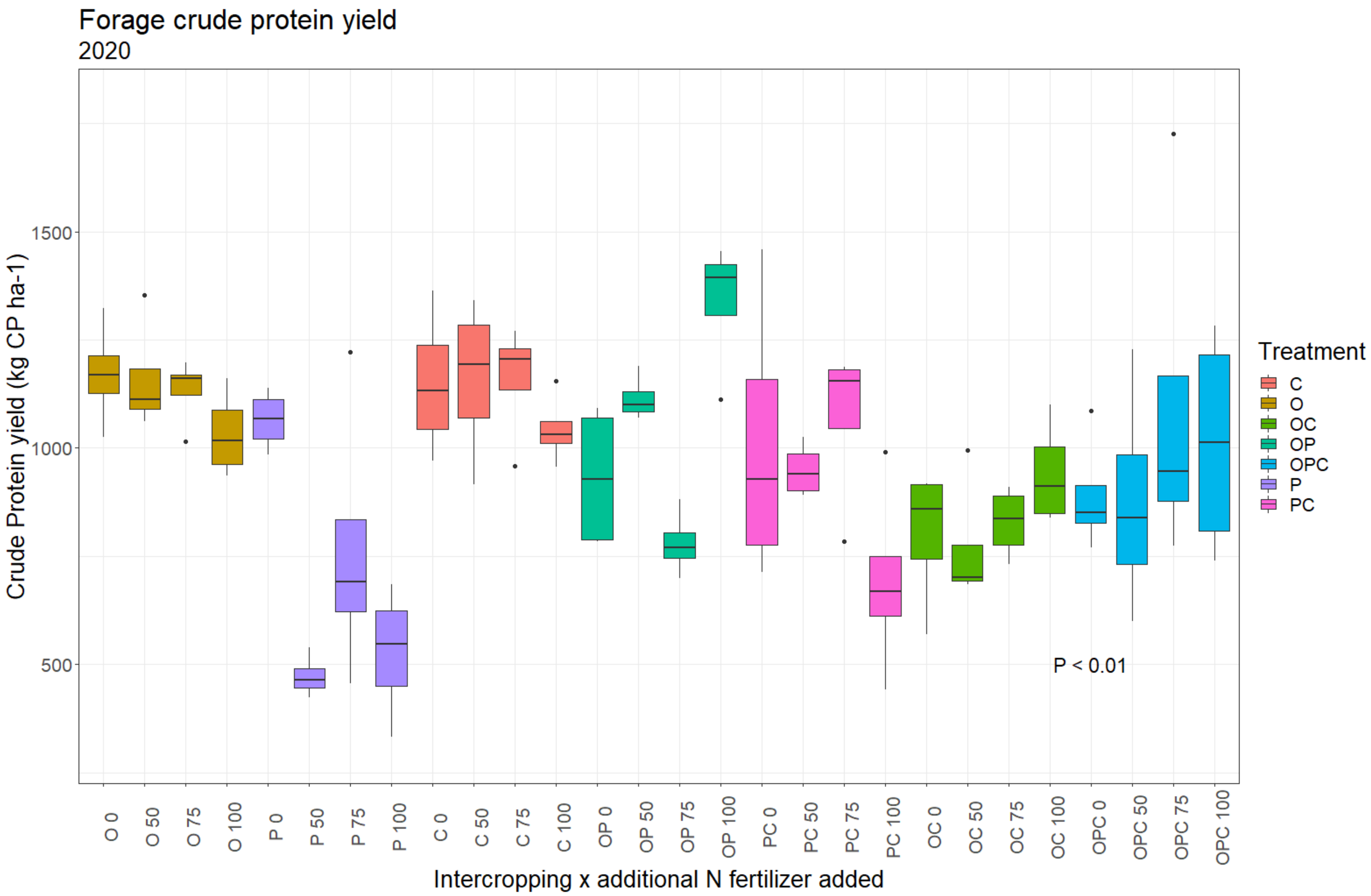

Forage crude protein yield
2020
Crude Protein yield (kg CP ha-1)
1500
1000
500
O 0
O 50
O 75
O 100
P 0
P 50
P 75
P 100
C 0
C 50
C 75
C 100
OP 0
OP 50
OP 75
OP 100
PC 0
PC 50
PC 75
PC 100
OC 0
OC 50
OC 75
OC 100
OPC 0
OPC 50
OPC 75
OPC 100
Intercropping x additional N fertilizer added
P < 0.01
Treatment
C
O
OC
OP
OPC
P
PC

Fig. 2.2. Forage crude protein (CP) yield calculated as biomass (kg ha^{-1}) in 2020. In 2020, CP yield decreases in P as additional N fertilizer is added. Furthermore, it seems that additional N fertilizer can promote increased CP yield in intercropping treatments in 2020. [2]

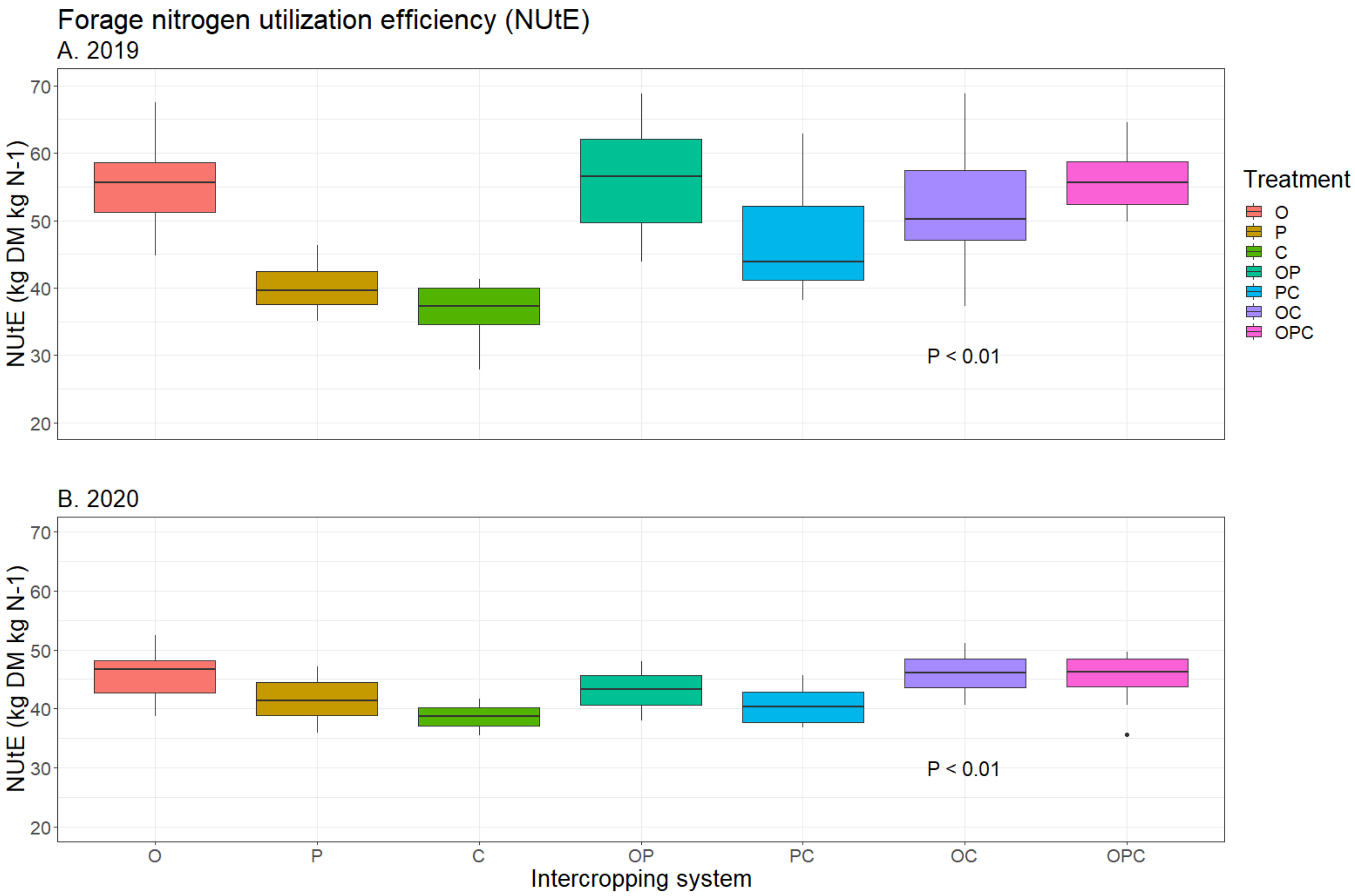

Forage nitrogen utilization efficiency (NUtE)
A. 2019
B. 2020
NUtE (kg DM kg N-1)
70
60
50
40
30
20
P < 0.01
P < 0.01
Treatment
O
P
C
OP
PC
OC
OPC
Intercropping system

Fig. 2.3. Nitrogen utilization efficiency (NU_tE) of intercropping systems in 2019 and 2020. In 2019 (A), we saw clearly that O, OP, OPC, and OC have relatively the highest NU_tE. While this is not as visible in 2020 (B), we were still able to see visually that C had the lowest NU_tE.[3]

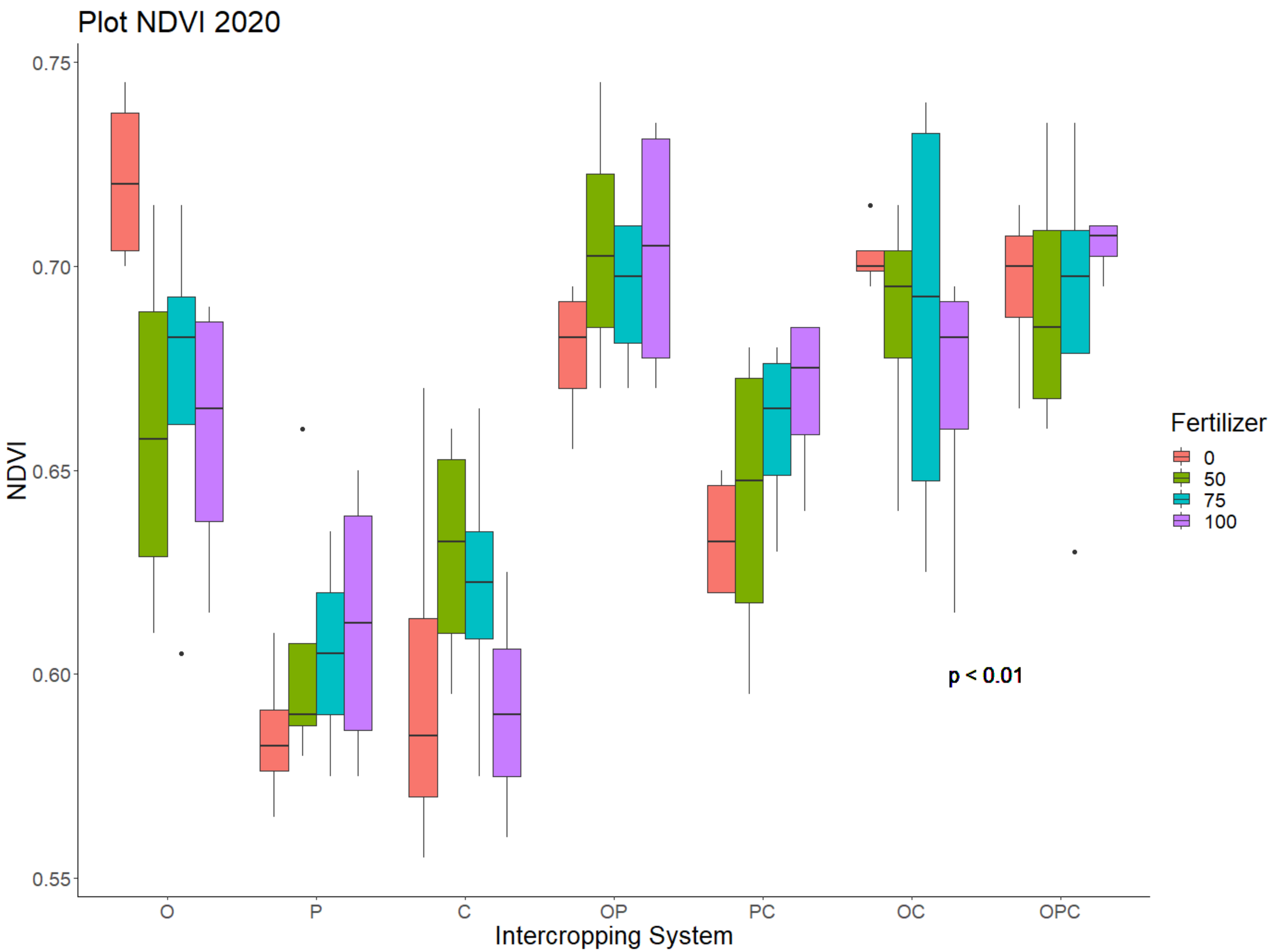

Plot NDVI 2020
0.75
0.70
0.65
0.60
0.55
NDVI
O
P
C
OP
PC
OC
OPC
Intercropping System
Fertilizer
0
50
75
100
p < 0.01

Fig. 2.4. Normalized difference vegetation index (NDVI) readings taken from the Greenseeker on 14 Aug. 2020. OP, OC, and OPC produced higher NDVI than O in general. PC produced higher NDVI than both P and C. While NDVI is a practical indicator of productivity, it is difficult to translate it into biomass yield (Fig. 2.1) or forage quality (Table 2.3, Table 2.4)[4]

Chapter 3: Oat-pea and pea-canola intercroppings with and without nitrogen fertilization alter biomass and crude protein water use-efficiencies

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3.1 Abstract

Intercropping can have both positive and negative effects on water use efficiency (WUE), and WUE can be also altered by nitrogen fertilizer addition. This study examined the effects of intercropping oats (O), peas (P), and canola (C) with and without N fertilizer addition on WUE. We measured changes in volumetric soil water content continually from seeding to harvest as well as crop biomass and crude protein (CP) productivities (i.e., CP yield) over two growing seasons. In 2019, where the rainfall was below long-term normal, intercropping options significantly impacted biomass WUE (WUE_{BM}) as well as WUE of CP (WUE_{CP}). Conversely, in 2020, when the rainfall was above normal, the differential margins between cropping systems narrowed. Specifically, while oat-pea intercropping (OP) showed better WUE_{BM} than P alone in 2019, this advantage disappeared in 2020. For instance, OP versus P alone showed a difference of 14.7 kg dry matter mm^{-1} in 2019 in contrast to a difference of only 0.7 kg dry matter mm^{-1} in 2020. Pea-canola intercropping (PC) improved WUE_{BM} consistently in both growing seasons and also had an advantage over P in WUE_{CP}; PC vs. P alone showed differences of 0.6 kg CP mm^{-1} in 2019 and 1.4 kg CP mm^{-1} in 2020. N fertilizer addition did not affect WUE_{BM} in the drier

2019, but it significantly decreased WUE_{BM} in the wetter 2020. This increase in crop water uptake in 2020 in fields receiving N fertilizer supports that increased availability in water and N resources does not consistently translate into improved biomass or CP yield as shown by diminishing WUE in the assessed intercropping combinations. Further research can focus on refining the choices of crop species combinations and their spatio-temporal arrangements to improve overall WUE in intercropping systems.

Keywords

WUE, Intercropping, oat, pea, canola, N fertilizer

3.2 Introduction

As the global average temperature is rising with escalating climate change, weather extremes are predicted to manifest more frequently (Jia et al., 2019). Water is a highly valuable resource for human society as it is used in manufacturing, energy generation, agriculture, and most importantly, daily living. With the growing population, the demand for water in each sector increases (FAO 2011). The unpredictable weather becomes a major challenge in water management as available water each year becomes less stable. Furthermore, the human population increase can lead to an increase in water pollution (Hendricks et al., 2019; Liang et al., 2021; Qiang et al., 2020;).

Additionally, although forage crops are often responsive to N fertilizer addition, overfertilization can be a major source of water pollution through leaching, runoff or gaseous losses (Hendricks et al., 2019; Liang et al., 2021; Qiang et al., 2020). In dry soil conditions, added N fertilizer could also be lost to the atmosphere via volatilization also compromising N availability for crop growth and diminishing productivity. Given the global pressure for increasing food production, it is therefore important to optimize N fertilizer input in consideration with underlying soil moisture availability (Thilakarathna et al., 2020). Overall, optimizing N fertilization management resides at the core of the dilemma between environmental conservation and food security (Chai et al., 2020).

Numerous studies have recommended intercropping systems as a means to sustain land productivity and enhance resilience (e.g., Chai et al., 2014; Qiang et al., 2020; Qin et al., 2018). Under a limited water supply, maize-soybean intercropping delivered higher productivity than the corresponding monocrops (Qin et al., 2018). As well, intercropped systems can also generate higher WUE than their monocrop counterparts (Caviglia et al., 2004; Gao et al., 2009; Ren et al., 2016). This attribute may translate into relatively better WUE performance of intercropping in drier climates, and hence intercropping can become suitable to buffer losses of crop productivity under environments with high risk of drought.

While existing studies explored the effects of reducing the water input in irrigated croplands (Brauman et al., 2013; Davies et al., 2017; Qin et al. 2018), little is known about WUE of forage cropping systems without any irrigation. In semi-arid regions such Western Canada, water supply mostly depends on seasonal rainfalls and the initial soil moisture storage at the beginning of the growing season. For instance, agriculture in Northern Alberta is primarily rainfed and over short growing seasons that experience variable moisture availabilities (Omokanye et al., 2021). Hence, it becomes useful to evaluate land productivity and water use across multiple cropping and N fertilization options. This study examined how N fertilizer addition and intercropping systems involving oats (O), peas (P), and canola (C) affect WUE of biomass (WUE_{BM}) and crude protein (WUE_{CP}) yields.

3.3 Methods

3.3.1 Site Description and Experimental Design

Field experiments were carried out over two growing seasons – from 30 May to 30 Aug. 2019, and from 22 June to 21 Sep. 2020, at the Fairview Research Farm, located in north-western Alberta, Canada (56°04'53.3"N, 118°26'25.1"W). The study was conducted using new plots each experimental year. This region experiences long and cold winters and short and mild summers, hence characterized as a boreal climate. The soil at the experimental site is classified as an Eluviated Black Chernozem, according to the Canadian System of Soil Classification (Agricultural Region of Alberta Soil Inventory Database AGRASID; Government of Alberta, 2020). The management history of the experimental sites was a long-term alfalfa stand for hay production until termination with Roundup WeatherMax® herbicide (glyphosate) the fall of 2018 (September) before the experiments commenced.

The long-term temperature and rainfall averages, as well as the observed temperature and rainfall for 2019 and 2020 were acquired through the Alberta Climate Information System (ACIS 2020) from a permanent weather station located on site (Table 1). Baseline soil sample collection and analyses were done before crop seeding in both years. Soil availabilities of nitrogen (N) and phosphorus (P) were deficient (Table 1). Upon alfalfa stand termination in fall 2018, the field was deep plowed, disced and harrowed. The sites were lightly harrowed before seeding operations.

The three plant species chosen for this experiment were oats (*Avena sativa* L.*,* O), peas (*Pisum sativum* L.*,* P), and canola (*Brassica napus* L., C), and were intercropped as oat-pea (OP) and pea-canola (PC). Seeding rates had the plant density targets of 300 plants m^{-2} for monocrop oats, 90 plants m^{-2} for monocrop peas, and 76 plants m^{-2} for monocrop canola. For each of the two intercropping systems, each plant species was seeded at 75% of their common seeding rates.

A factorial randomized complete block design was implemented, where the two factors were intercropping systems (O, P, C, OP, PC) and nitrogen fertilizer addition rates of 0 and 75 kg N ha^{-1} (0N, 75N) with four block replicates. On May 30, 2019 and June 22, 2020, we used a 6-row small-plot seeder (equipped with disc-type openers) with a 22.9 cm row spacing to seed into 2 m by 16 m plots, and the base fertilizer (a mixture of P, K, and S) and N fertilizer addition (granular urea, 46-0-0) were banded at seeding (Table 1). To reduce weed competition, we applied glyphosate (StartUp® Roundup) herbicide as pre-emergent in 2019 and pre-pass in 2020 as well as hand occasional weeding.

3.3.2 Plant sample collection and measurements

In 2019, forage was manually harvested (within 14 to 22 Aug.), while in 2020 (within 31 Aug. to 1 Sept.), a 63.5 cm wide custom-made forage harvester (Swift Machine and Welding Ltd. (Swift Current, Saskatchewan, Canada) was used for mechanically harvesting the forage. These harvests were done in the 3 inner rows (at least 2 m in length) of each experimental plot. The outer rows were not considered in these harvest measurements to avoid edge effects. The crops were cut leaving an approximate 10 cm stubble. The fresh forage materials were sub-sampled (750-1000 g), dried and weighed to determine dry matter content, which was used to calculate forage biomass yield on dry matter basis. Forage samples from both 2019 and 2020 were sent to A&L Canada Laboratories (London, Ontario, Canada) for assessing crude protein (CP) content using Dumas dry combustion method in a LECO FP628 nitrogen analyzer (AOAC 990.03, 2005) that determines total N content, where CP was calculated as %N x 6.25 (AOAC 1984).

Water use efficiencies focusing on biomass (WUE_{BM}) or CP (WUE_{CP}) yields were calculated as:

$$WUE_{BM} = \frac{Forage\ dry\ matter\ biomass\ yield}{ET} \quad [1]$$

$$WUE_{CP} = \frac{Forage\ CP\ yield}{ET} \qquad [2]$$

where the forage CP yield was calculated as the product of the CP content and the dry biomass yield.

Evapotranspiration (ET) is conceptualized as:

$$ET = P + I + U - R - D_w - \Delta S \qquad [3]$$

where rainfall (P) was acquired through the ACIS (2020) weather station as the seasonal accumulation between seeding and harvest dates. As the plots were not irrigated, irrigation (I) was omitted from the equation. Runoff (R) was assumed to negligible at the experimental sites because of the flat topography (<2% terrain slope). The upward (U) and downward (D_w) flow of water were also assumed to be negligible based on Darcy's law (Gao et al., 2009; Kar et al., 2007; De Medeiros et al., 2005). The change in soil water storage (ΔS) over the season was calculated using measurements of volumetric water content (VWC).

The VWC was recorded in 30-minute intervals throughout the growing season using 5TM moisture sensors interfaced with EM50 dataloggers (Meter Group, Inc., USA). The soil sensors were installed at crop interrow positions at depths of 15 and 30 cm to represent and capture soil moisture contents within the soil layers of 0-22.5 (topsoil) and 22.5-37.5 cm (subsurface), respectively. The VWC data were normalized separately as daily averages from 0:00:00 to 24:00:00 of each day within each growing season and for each experimental plot and soil depth. Water content daily patterns were visualized for each soil depth, experimental treatment, and year separately (Fig. 1). VWC was converted from fractional values into water column (mm) by multiplying the measured VWC by the soil depth ranges of 22.5 cm (0-22.5 cm topsoil layer) and 15 cm (22.5-37.5 cm subsurface layer).

The seasonal change in soil water uptake (ΔS) was estimated by subtracting moisture present at the end of the growing season from the moisture at the beginning of the season in each experimental plot and soil layer. For further examination of temporal patterns, each growing season (i.e., 2019 and 2020) was broken into two major intervals. The intervals within the growing season 2019 consist of i) the first major rainfall interval and the ii) second major rainfall interval. The periods within the growing season 2020 consist of i) the early period before the major rainfall and ii) the period after the major rainfall. For each experimental plot and soil layer, soil water uptake was estimated by subtracting moisture present at the end of the period from the moisture at the start of the period.

Because of sensor malfunctioning leading to missing data, the available data was subject to multiple imputation using the MICE package (van Buuren and Groothuis-Oudshoorn, 2011) with the aim of developing a complete dataset for further complete analysis. In 2019, 25% of the overall data was generated using imputation, and in 2020, only 17.5% of the data in the 0-22.5 cm layer and 32.5% of the data in the 22.5-37.5 cm layer were generated using this imputation approach. Once this gap filling procedure generated a completed dataset, the resultant VWC by the 0-22.5 and 22.5-37.5 layers were added to create the ΔS for the entire 0-37.5 cm soil profile.

3.3.3 Statistical Analysis

Data was processed through the R statistical program (R Core Team 2020) at alpha critical value of 0.05, using the NLME program (Pinheiro et al. 2020) to complete two-factor analyses of variance (ANOVA) for the WUE and the crop water uptake results. ANOVA included cropping options and N fertilizer addition as fixed factors as well as block replicate as a random factor. A pairwise comparison post-hoc test was applied to ANOVA tests which found significant differences using the emmeans package (Lenth, 2020).

3.4 Results

Total rainfall over the crop growing season 2019 was lower than the long-term normal (Table 1). Both Jun. and Aug. 2019 had rainfalls slightly higher than the long-term monthly averages, while Jul. and Sep. had monthly rainfalls much lower than the long-term averages. Conversely, the crop growing season 2020 experienced a cumulative rainfall larger than the long-term normal (Table 1). July 2020 experienced a cumulative monthly precipitation higher than the long-term normal by 20 mm.

In further details, two rainfall intervals mainly influenced the soil moisture contents in 2019 (Fig. 1A), while one single major rainfall changed soil moisture patterns in 2020 (Fig. 1C). The major rainfall in the middle of the crop growing season 2020 had a sizable magnitude of 28.3 mm (on 3 Aug.), which combined with a high initial soil moisture storage at the beginning of the growing season in 2020 made 2020 overall much wetter than 2019.

During the first major rainfall interval of 2019, soil water did not change at the topsoil layer (0-22.5 cm), but a significant interaction effect ($p = 0.04$) of intercropping with N fertilizer addition was observed at the subsurface layer (22.5-37.5 cm) (Table 3). Specifically, only canola alone at 0N (C0, Fig. 2) exhibited a significantly larger decrease in soil moisture compared to the rest of the treatment combinations in the study. Following the second major rainfall interval of 2019, soil water storage was similar across treatment combinations at both soil layers (Table 3).

Before the major rainfall of early Aug. 2020, no significant differences in soil water storage across any treatments were observed at the topsoil layer (0-22.5 cm), but at the subsurface layer (22.5-37.5 cm), treatment effects were indeed observed (Table 3). O at 75N had the greatest decrease in soil moisture storage before the major rainfall with a change of -13.0 mm (Fig. 3A). While not statistically significant, every intercropping system apart from P had a numerical increase in soil water storage as a response to the addition of N fertilizer (Fig. 3A). After the major rainfall event, no significant difference in soil water storage was observed at the topsoil layer, but the subsurface layer did show treatment effects (Table 3). Similar to the results prior to the major rainfall in early Aug. 2020, intercropping systems (apart from P) seemed to experience increased water use with increasing N fertilizer rate (Fig. 3B).

WUE_{BM} was significantly different between intercropping systems in both years (2019, $p < 0.01$; 2020, $p < 0.01$), and between N fertilizer rates in 2020 ($p < 0.01$) (Table 2). O was the most water use-efficient system in 2019, with 12 kg dry matter mm^{-1} above the second highest intercropping system (OP; Table 4). While OP and PC performed significantly better than P alone in terms of WUE_{BM}, P performed similar to C, where the WUE_{BM} of both OP and PC were only slightly higher than C. In 2020, O was also the most water use-efficient system, while the other intercropping systems in 2020 did not differ from each other ($p > 0.05$) (Table 4). 0N had better WUE_{BM} than 75N in 2020, by a difference of 8 kg dry matter mm^{-1} (Table 4).

The WUE_{CP} greatly differed across intercropping systems within both 2019 and 2020 (Table 2). In both 2019 and 2020, OP was one of the poorest performing systems, while C was one of the top performing systems in terms of WUE_{CP} (Table 4). Conversely, PC had poor WUE_{CP} in 2019, but had relatively similar WUE_{CP} in 2020 to O and C. Compared to 2019, WUE_{CP} in 2020 were relatively similar between intercropped systems.

The seasonal change in soil water storage was statistically unaffected by experimental treatments in both 2019 and 2020 at the topsoil layer (0-22.5 cm); however, at the subsurface layer (22.5-37.5 cm), intercropping systems differed from each other in 2019 and the impact of N fertilizer addition was seen in 2020 (Table 2). In 2019, C, O, and OP have the largest soil moisture change (as attributable to differences in crop water uptake), and significantly higher than P and PC by approximately 3 mm (Table 4). In 2020, 75N rate showed a larger soil moisture removal than 0N by 1.5 mm (Table 4).

In both experimental years, the temperature during the growing season was very similar to the long-term normal (Table 1). The soil temperature at seeding was on average 15.6 ℃ in 2019, and 23.44 ℃ in 2020.

3.5 Discussion

The biomass WUE (WUE_{BM}) comparisons in our study were very similar in the two growing seasons, but with more moisture available during the growing season 2020, the differences in across intercropping systems narrowed, while the differences due to N fertilizer addition widened. The source of differences across the two study years came mostly from pea alone, which showed a significant WUE_{BM} difference between the two years. Peas are sensitive to moisture stress at flowering and pod formation stages (Mishra and Singh, 2002), and this explains the poor WUE_{BM} as the rainfall events occurred mostly early in the season, particularly in 2019, before the reproductive stages occurred in our study. In terms of plant behavior across the two study years, WUE_{BM} increased with greater moisture available in 2020 in P, C, and PC treatments, while WUE_{BM} actually increased with lesser moisture available in 2019 in O and OP. This latter effect observed in O and OP has been reported in previous studies, where increased water input in the form of irrigation in these oat systems (O, OP) negatively impacted WUE (Feng et al., 2017; Ye and Li, 2009). Conversely, the former effect of increased WUE_{BM} in P, C, and PC cropping systems with greater moisture available has not been previously reported in the literature, and more research can further focus on these responses. Collectively, these overall results indicated that different intercropping systems produce different advantages, directly depending on the selection of crop species and their combinations. As found in our study, canola alone and pea alone produced a better WUE with increasing moisture content, whereas oat alone remained consistent irrespective of drier vs. wetter seasons. Moreover, the combination of pea and canola replicated these effects of the crops planted alone, while the combination of pea and oat did not. Our study provides these WUE insights of novel intercropping forage systems for the first time in literature. Based on the collected soil moisture data, results further suggest that the reason for these interactions is predominately driven upon the different plant root structures as well as their mechanisms for water uptake.

As expected, the forage CP content was generally high in canola and pea at silage harvest stage (Lee et al., 2021). In terms of CP content per unit of biomass, pea and canola were similar and actually both outperformed oat. However, this study shows that in terms of efficiency, peas are sub-ideal at converting water uptake efficiently into CP production. Conversely, canola excels at this process. The poor WUE_{CP} in OP could be explained as competition between the two plant species. OP could be sacrificing CP gains as a means to maintain adequate biomass yield, thus manifesting a dilution effect (Jarrell and Beverly, 1981). Since both WUE_{BM} and WUE_{CP} decreased when intercropping oats with peas, it is possible that these plants are expending proportionally more energy in amassing resources such as water and nutrients than in producing biomass and crude protein gains. Within the PC system, the plants are likely not competing interspecifically as their taproots are predominantly vertical in the soil profile with proportionally less horizontal root growth, thus there could be less competition for water between peas vs. canola compared to the fibrous oat roots that grow more horizontally.

As abovementioned, we identified differences in water uptake across experimental treatments in 2019 (Table 4). Across the three sole crops, we found that oat and canola overall have similar water uptake patterns, while pea has a lower uptake. By this observation, the initial assumption was that the combination of these species should yield a mixed compromise between the behavior of the sole crop's uptake. However, the results indicated that OP was even more effective at water uptake than O and P, and PC actually performed exactly like P, thus poorer than C. A previous study mentions that legumes and oilseed plants require less water when intercropped with cereals (Chai et al., 2014). However, our study demonstrates that rather than decreased water requirements, intercropping oat with pea improves water accessibility in the soil and uptake, as more water was removed from the soil than the sole crops, given the same amount of water input over the growing season across all cropping systems. This could indicate that fibrous root systems (e.g., oats) can work synergistically with taproot systems (e.g., peas) to maximize water uptake (Inanaga et al., 2002). Conversely, the presence of two taproot plants into an intercropping system (e.g., canola and peas) did not improve water uptake from the soil profile (including both 0-22.5 and 22.5-37.5 cm layers). Therefore, one could conceptualize that this possible synergistic relationship between fibrous and taproot systems of oat with pea in the present study, which improved water uptake, reveals the positive effects of plant species diversity within an intercropping system on ecosystem services. Future research efforts are needed to further understand how belowground growth drives productivity-WUE relationships.

By comparing the moisture uptake data across the two years, it became self-evident that soil moisture is a driving factor to nitrogen use, as water uptake between intercropping systems no longer significantly differed in the presence of abundant water (in the growing season 2020), and that water uptake significantly differed between with and without N fertilizer addition instead. This observation initially supports the notion of water being a hierarchically dominant factor of plant growth followed by nutrient availability. In terms of WUE in our study, PC resembled C (Table 4), but in terms of water uptake, PC resembled P (Table 4). While PC has similar WUE_{BM} as C, this entailed an increased water uptake to produce the same amount of biomass. Further studies could investigate options to improve water uptake in PC systems such as different P-to-C seeding ratios as well as relay or alternate row intercropping systems while also assessing different N fertilizer management choices.

In the wetter 2020, when we witnessed a significant difference between N fertilizer rates ($p < 0.01$, Table 4), the increased water uptake from increasing N fertilizer rate led to an overall decrease in WUE_{BM}. A potential reason is that N became a luxury consumption by plants upon N availability derived from both soil mineralization and urea addition (de Mazancourt and Schwartz, 2012). By controlling and capturing surrounding N resources, a cropping system can maintain the competitive edge over other plants growing in the vicinity, thus leading to an increase in water uptake and little biomass gain. That said, the metric for measuring plant productivity in the current study is plant aboveground biomass, and does not account for root mass, which would have further altered our current understanding of WUE.

When observing the water uptake in two separate periods within each experimental year, we notice that in the wetter year (2020), the demand for water differs between treatments in both subsequent periods within the growing season (Fig. 3), while in the drier season (2019), the difference ceases to exist over the second major rainfall interval (Table 2, Fig. 1). This could be explained by the physiological growth of plants under drought stress. In general, plants require more water during the reproductive phase for heading and grain filling (Hussain et al., 2016, 2019; Rosa et al., 2020; Zeleke and Nendel, 2019), which should lead to an increase in plant water usage over the later part of the growing season 2019. We did not detect this increasing plant water usage as the soil layer depths within which the moisture sensors were installed had been already depleted of available water. As the early growth period in 2019 experienced heavy rainfalls, the root growth may have even exceeded the soil depths of the sensor placements and acquiring water at deeper soil layers to maintain high water consumption (Hussain et al., 2019). With an addition of nitrogen fertilizer, more energy can be expended in overall plant growth, thus an increased demand for water.

Within the two sequential periods over the growing season 2020 (i.e., before and after the major rainfall in early August; Fig. 1C), we observed different responses of crop water uptake with vs. without N fertilizer addition on different intercropping systems. Sole oat consistently removed more water from the soil with added N fertilizer (75N). Sole pea did the exact opposite, where the N fertilizer addition decreased plant water uptake throughout the period of active crop growth. Sole canola with N fertilizer addition removed less water from the soil during the first half of the growing season, but more water later in the subsequent growth period. This phenomenon could be explained by the physiological growth pattern each plant has during a growing season. Oat development is considered early and fast relative to other crops, and hence oat requires comparatively more water earlier on to support its development (Muller et al., 1984). With the stimulating addition of N fertilizer, more energy can be expended for plant growth, thus an increased demand for water. Conversely, with peas, N addition seems to partially inhibit water uptake from the soil. One possible speculation is that as a legume, peas do not need as much water uptake for maintenance and growth when in the presence of sufficient accessible nutrients than when compared to needing the support of the rhizobia infestation on pea roots and their biological N fixation. While this study did not observe significantly different nodulation performances with vs. without N fertilizer addition, past studies have shown that with N fertilizer addition, peas have decreased nodulation performance (Clayton et al., 2004). As N fixation consumes a significant amount of energy, metabolic replenishment of this energy would also require a significant water use, thus probably leading to the increased water consumption from pea plants. Conversely, the water use by canola could have been impeded by high salt content in the soil in the early stages of growth. As we found that canola with 0N had better WUE than 75N in 2020, this correlates well with how canola at 75N uptake more water after the major rainfall that occurred in the middle of the growing season.

In general, intercropping seems to change plant water uptake as a function of N fertilizer addition, such that the behavior found in sole crops was different from when intercropped. In both 2019 and 2020, addition of N fertilizer positively influenced water uptake in intercropped systems. Relative to monocrops, one plausible explanation to this change is due to the increased seeding rate in the intercropped fields. However, there is also the possibility for a synergistic interaction between plant species that caused the increased water uptake collectively. Such interactions between plant species could include competition for the added N fertilizer, thus the need for more water. Another plausible mechanistic interaction could be roots of different plants operating together to increase water access and usage within the rhizosphere. This interaction is often mentioned in the literature when discussing cereal-legume intercropping systems because the taproot from legumes could facilitate the fibrous roots of cereals to grow and reach deeper layers of the soil profile, and hence, this can provide more access to moisture (Bargaz et al., 2016; Li et al., 2006). However, in an intercropping system with two taproot plants, increased competition or no interaction are more likely. That said, intercropping could also be alleviating the "N inhibition", the effect of inhibiting nodulation under the effect of N fertilization, in pea plants in plots with N fertilizer addition (Hu et al., 2017; Zhao et al., 2016). By intercropping, pea nodulation is promoted to compete with the other intercropped plant in the mixture, whether it is oat or canola.

Certain limitations of this field study can be noted. Soil sensors were deployed at two depths in all experimental plots (i.e., 15 and 30 cm), and as noted above, some of the crops could have extracted water from deeper soil layers in particular in the mid and late growing season, and such this differential deep water uptake would have been unaccounted for in our study. Likewise, the surface soil could have dried out much quicker as a response to evaporation particularly during the early growing season, and hence, the sensor placed at the 15 cm depth might have misrepresented the water content right at the surface soil. Additionally, any difference in spatial redistribution of water rainfall by the distinct canopy architectures of the crop species (Logsdon et al., 2010; Singer et al., 2010) was also unaccounted for in our study because all soil sensors were installed in the crop inter-rows with the aim of preventing damage to the emerging seedlings in the early growing season.

3.6 Conclusion

This study showed how common monocrop and novel intercropping systems for forage production influence WUE and how their effects are amplified in drier growing seasons. Intercropping options do not lead to WUE superior to oats alone, but intercropping pea-canola did provide WUE higher than that of canola alone or pea alone. In general, WUE is determined by the plant's ability to translate water uptake into yield, where water uptake was governed by seasonal rainfall and N fertilizer addition. However, this study showed that each crop species and intercropping combination has a distinct uptake behavior over contrasting growing seasons and soil layers. Under high soil moisture conditions, N fertilizer addition becomes more significant to increase water uptake compared to the influence of crop species selection. Nevertheless, under future climate change scenarios with an increased likelihood of drought, wherein N fertilization could exert an even lesser effect on WUE than cropping system type, additional investigation could proactively inform how to further optimize the selection of crop species combinations that can deliver beneficial WUE thereby sustaining plant productivity and resilience within intercropping systems.

3.7 Acknowledgements

We would like to thank Liisa Jeffrey, Kaitlin McLachlan, Johanna Murray, Buthaina Al-Maqtari, Dong Hyeon Kim, Courtney Chaykowski, and Frances MacArthur for the continued support throughout the project. This research was financially supported by the Canadian Agricultural Partnership (CAP) - Environmental Stewardship and Climate Change Group Program (ESP-048569).

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3.9 Tables and Figures

Table 3.1. Rainfall, temperature, and soil properties (0-15 cm depth increment) at the experimental site, as well as the basal fertilizer applied in 2019 and 2020[5]

	2019	2020	Long Term Average
Month	Rainfall (mm)		
June	72.9	67.2	64.5
July	61.9	89.8	69.5
August	49.1	53.9	47.5
September	24.6	23.1	33.7
Total	208.5	234.0	215.2
	Air temperature (°C)		
June	14.1	14.0	14.0
July	15.1	15.6	15.9
August	12.9	14.1	14.6
September	9.7	10.3	9.6
Soil property			
Nitrate-N (mg kg^{-1})	5	24	
P (mg kg^{-1})	7	10	
K (mg kg^{-1})	242	216	
S (mg kg^{-1})	5	5	
Ca (mg kg^{-1})	1920	1760	
Mg (mg kg^{-1})	354	359	
pH	6.2	6.2	
Organic matter, OM (%)	8.2	3.7	
Fertilizer Blend Applied			
P_2O_5 (kg ha^{-1})	27.2	10.9	
K_2O (kg ha^{-1})	27.2	Nil*	
S (kg ha^{-1})	12.7	13.6	

* Soil tests indicated adequate availability of soil K, so no additional K_2O was applied.

Table 3.2. ANOVA p-values of crude protein (CP) and biomass (BM) water use efficiency (WUE) and seasonal water uptake of plots under different intercropping systems and N fertilizer addition.[6]

	WUE_{BM}		WUE_{CP}		Seasonal water uptake			
					2019	2019	2020	2020
	2019	2020	2019	2020	0-22.5 cm	22.5-37.5 cm	0-22.5 cm	22.5-37.5 cm
Intercropping (I)	<0.01	<0.01	<0.01	0.01	0.12	<0.01	0.24	0.40
Fertilizer (F)	0.96	<0.01	0.97	0.09	0.98	0.08	0.14	0.04
I x F[1]	0.98	0.27	0.71	0.40	0.35	0.37	0.46	0.08

[1] I x F: interaction between Intercropping (I) and Fertilizer (F)

Table 3.3. ANOVA p-values of water uptake within selected intervals of the 2019 and 2020 growing seasons under different intercropping systems and N fertilizer addition.[7]

	2019 (FR[1]=day 180-205, SR[2]=day 206-246)				2020 (BMR[3]=day 194-215, AMR[4]=day 216-241)			
	FR 0-22.5 cm	FR 22.5-37.5 cm	SR 0-22.5 cm	SR 22.5-37.5 cm	BMR 0-22.5 cm	BMR 22.5-37.5 cm	AMR 0-22.5 cm	AMR 22.5-37.5 cm
Intercropping (I)	0.42	<0.01	1.00	0.36	0.22	0.02	0.09	0.63
Fertilizer (F)	0.36	0.10	0.45	0.51	0.05	0.13	0.11	0.12
I x F[5]	0.37	0.04	0.59	0.26	0.42	<0.01	0.87	0.02

[1] FR: First rainfall interval, from 29 Jun. to 25 Jul. 2019

[2] SR: Second rainfall interval, from 25 Jul. to 3 Sep. 2019

[3] BMR: Before major rainfall event, from 12 Jul. to 2 Aug. 2020

[4] AMR: After major rainfall event, from 3 Aug. to 28 Aug 2020

[5] I x F: interaction between Intercropping (I) and Fertilizer (F)

Table 3.4. Water use efficiency (WUE) and seasonal water uptake. The WUE and water uptake of intercropping systems and N fertilizer applications are shown and compared separately. While adequate moisture affects biomass WUE (WUE_{BM}) and water uptake in terms of N fertilizer addition, it does not affect crude protein WUE (WUE_{CP}).[8]

	WUE				Seasonal Water Uptake (mm)			
	WUE_{BM} (kg dry matter mm^{-1})		WUE_{CP} (kg CP mm^{-1})		0 – 22.5 cm topsoil layer		22.5 - 37.5 cm subsurface layer	
Intercropping	2019	2020	2019	2020	2019	2020	2019	2020
O	68.1 a[1]	71.0 a	7.6 ab	9.6 a	18.2 a	23.5 a	6.9 ab	11.5 a
P	41.1 c	49.1 b	6.2 b	7.5 ab	15.9 a	22.1 a	4.5 b	9.3 a
C	51.1 bc	58.3 ab	8.6 a	9.3 ab	19.7 a	19.7 a	7.5 a	9.8 a
OP	55.8 b	49.8 b	6.2 b	7.3 b	18.9 a	24.9 a	7.5 a	9.7 a
PC	52.9 b	57.9 ab	6.8 ab	8.9 ab	16.9 a	27.0 a	4.5 b	10.3 a
N-Fertilizer Rate								
0N	53.9 a	61.4 a	7.1 a	8.9 a	17.9 a	25.0 a	6.6 a	9.3 a
75N	53.7 a	53.0 b	7.1 a	8.1 a	17.9 a	21.9 a	5.7 a	10.9 a

[1] Factors with same letters within a column indicate no significant differences based on Tukey HSD test

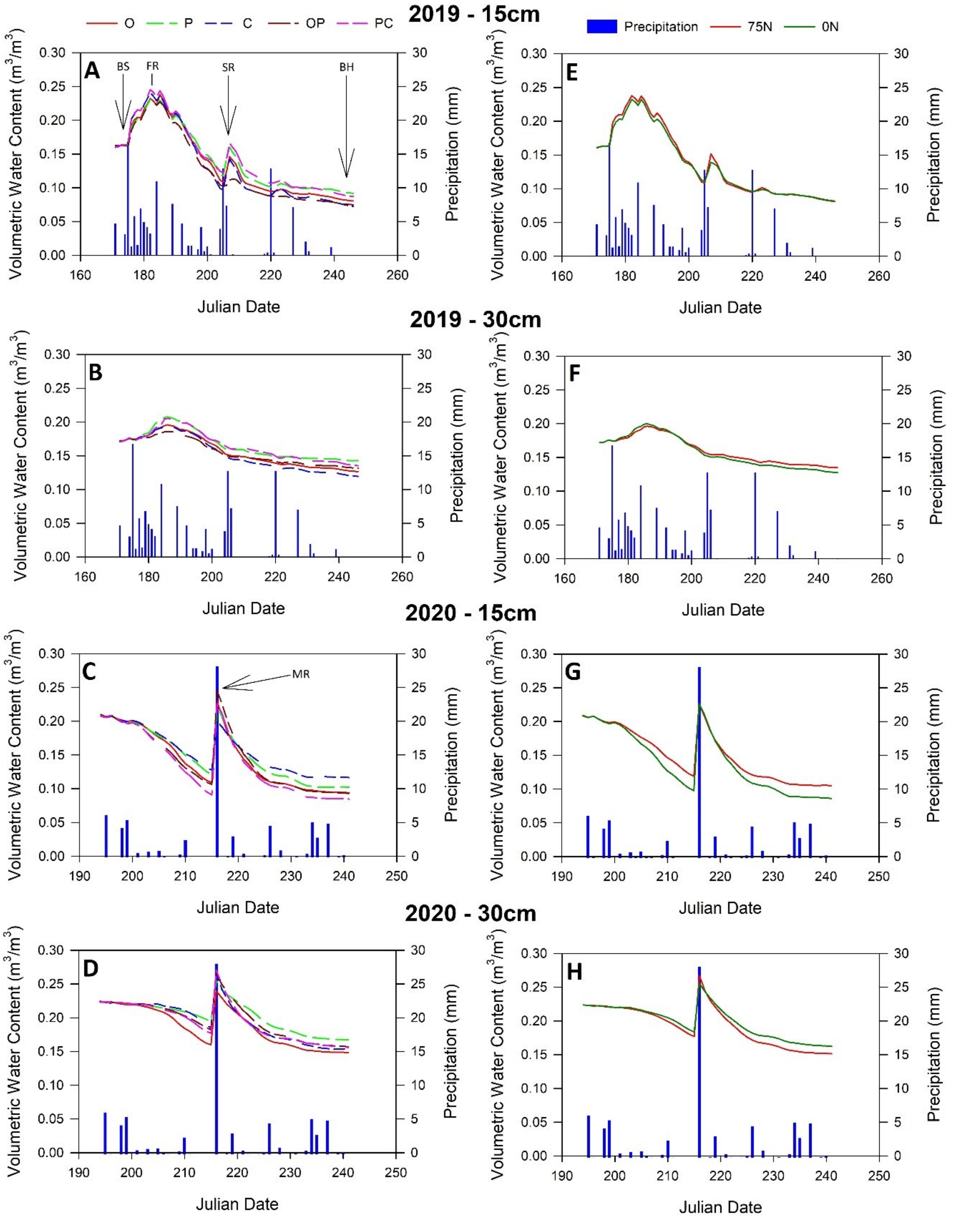

Fig. 3.1. Daily average moisture measurements at two soil depths (15 and 30 cm) throughout the growing seasons of 2019 and 2020 along with daily cumulative rainfall.

Upon observing the timings of rainfall compared to the moisture readings at the time, we defined two key periods within each experimental year. In 2019, the two key periods were: during the first major rainfall interval (Julian days 180-205), and during the second major rainfall interval (days 206-246). In 2020, the two key periods were: before the major rainfall event (days 194-215), and after the major rainfall event (days 216-241). BS: 1 week before seeding, BH: 1 week before harvest. FR: First rainfall, SR: Second rainfall, MR: Major rainfall[5]

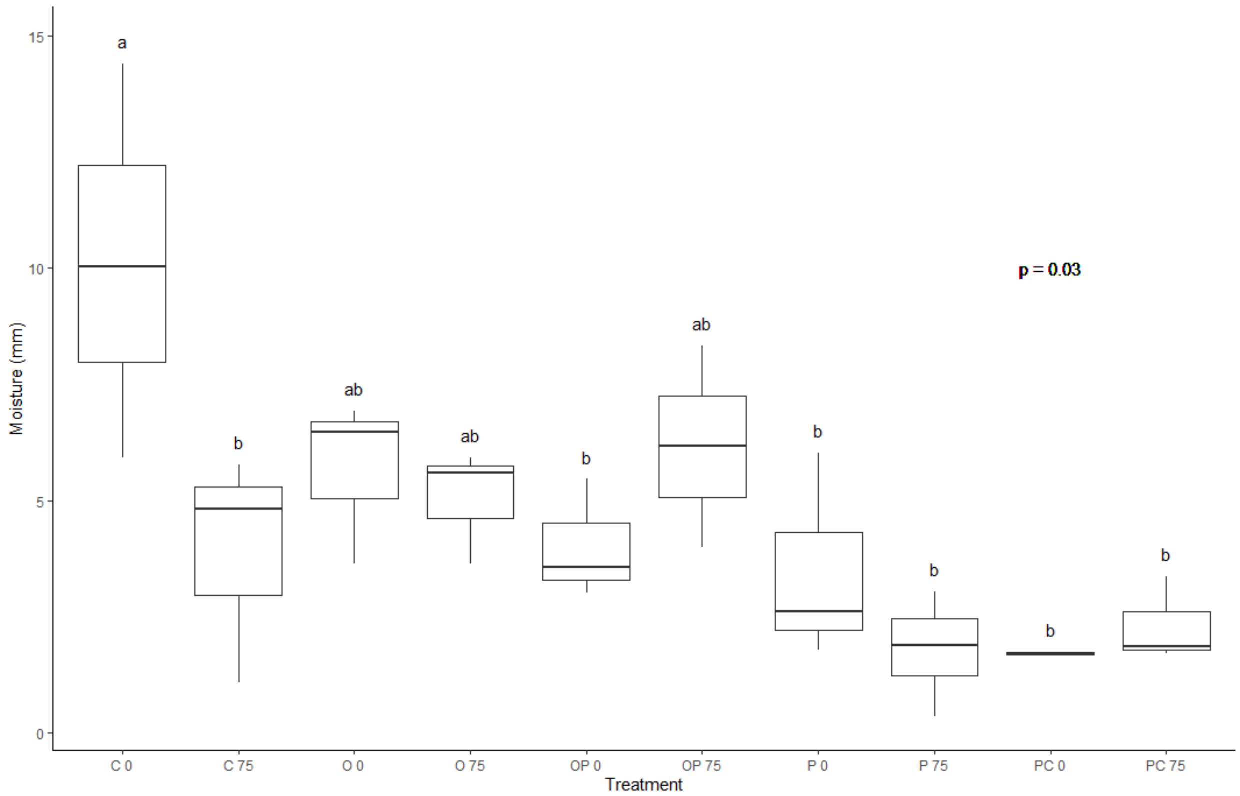

Fig. 3.2. Water uptake of treatment combinations (N fertilizer x intercropping systems) during the first major rainfall interval in 2019 at the 22.5-37.5 cm soil layer. C at 0N showed a high water uptake compared to the rest of the treatments. OP and PC had an increase in water uptake with the N fertilizer addition, while sole cropping systems exhibited decreases in water uptake. This demonstrates the efficiency of intercropping in sourcing soil water. Treatment combinations labelled with the same letters above the boxplot indicate that there are no significant differences based on Tukey HSD test.[6]

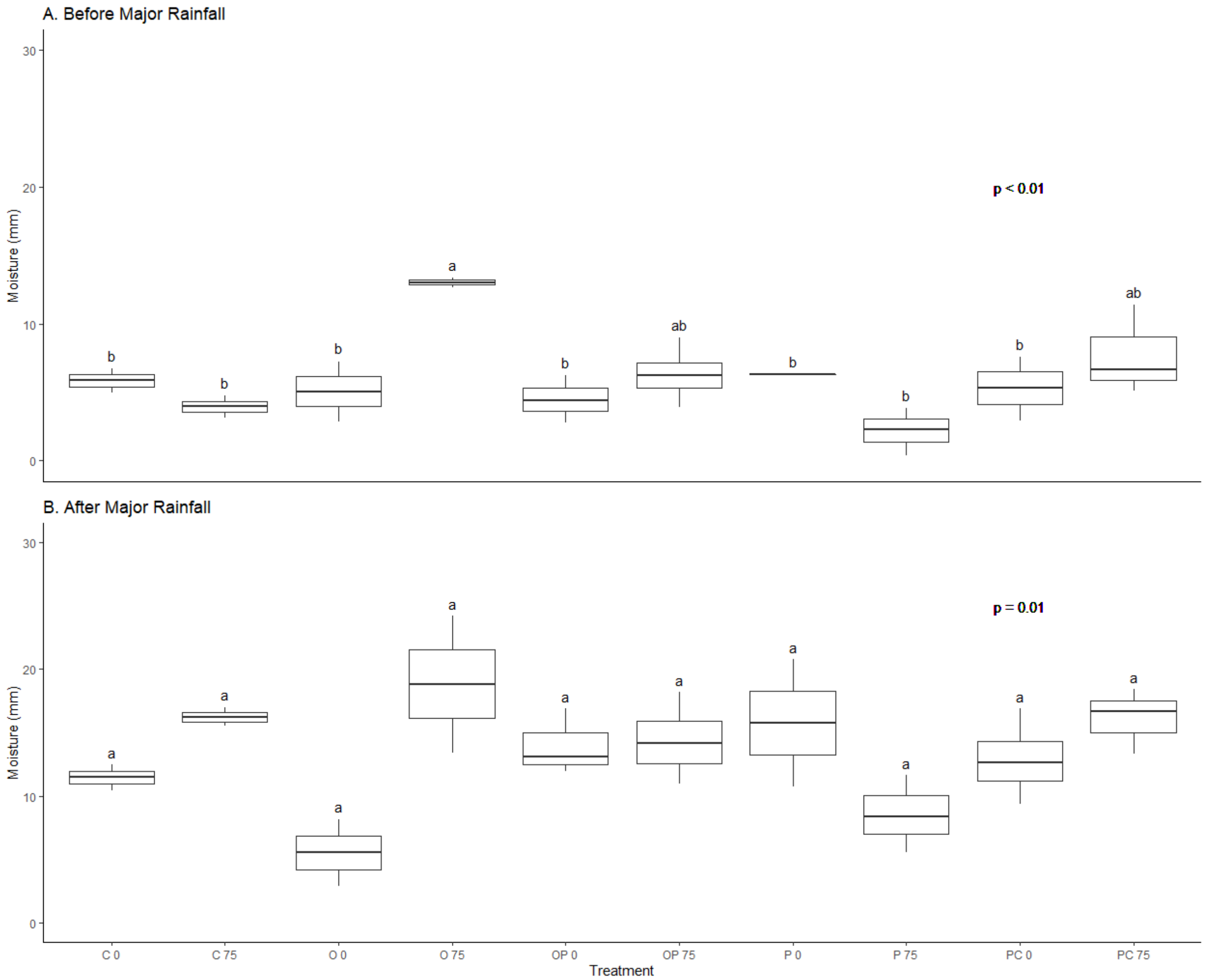

Fig. 3.3. Water uptake of treatments (N fertilizer x intercropping systems) at the 22.5-37.5 cm soil layer (A) before and (B) after the major rainfall in Aug. 2020. Before the major rainfall, C and P were the only two systems that decreased in uptake with additional N fertilizer. Similar to 2019 (Fig. 3.2) and prior to the major rainfall in Aug. 2020, P consumes less soil water in fields that received N fertilizer. This indicates that pea performs better without N fertilizer in terms of water uptake, where the other crops in the study were benefited by N fertilizer addition, in terms of water uptake. Water uptake of C increases with N fertilizer addition after the major rainfall event. Treatment combinations labelled with the same letters above the boxplot indicate that there are no significant differences based on Tukey HSD test.[7]

Chapter 4: Identifying key mycorrhizal genus in oat-pea intercropping: How mycorrhizal genera can help determine stressed plants and how N fertilizer affects mycorrhizal abundance

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4.1 Abstract

Arbuscular mycorrhizal fungal diversity can be altered by intercropping plant species, as well as N fertilizer applications. This study examined the effects of oat-pea intercropping and N fertilizer addition on the richness and diversity of mycorrhizal species, as well as identified the most common arbuscular mycorrhizal fungi (AMF) genera recruited for oats and peas in two growing seasons (2019 and 2020). The root AMF community was significantly different than the soil AMF community. The AMF diversity was higher in an intercropped system compared to their respective monocropping system. Under drier conditions in 2019, arbuscular mycorrhizal richness decreased with N fertilizer addition in sole peas and increased with N fertilizer addition in sole oats, but no significant change in richness was observed in oat-pea intercropping. During the wetter growing season 2020, arbuscular mycorrhizal diversity increased when oat and pea were intercropped, compared to either sole oat or sole pea. The presence of *Diversispora* mycorrhizal genus in sole pea was associated with a root community different than in sole oat.

Claroideoglomus richness increased in peas in 2020, thus this genus could be moisture dependent. *Paraglomus* richness in oat-pea intercropping was similar to sole oat in 2019, while similar to sole pea in 2020. This can suggest that *Paraglomus* is an indicator of plant stress under intercropping, as based on the premise that stressed plants release more exudates, and the subsequent mycorrhizal associations favor these plants with higher exudation. Future investigations can further reveal the functions and benefits of these mycorrhizal genera in annual monocrop and intercropping systems.

4.2 Introduction

Intercropping can provide many benefits. Certain intercropping options have demonstrated overyielding capacity (Xiao et al. 2019, Wang et al. 2017) or yield stability under contrasting environments (Lee et al. 2021, Petrova Chimonyo et al. 2019, Weih et al. 2021). Furthermore, intercropping forage systems have delivered consistently better nutritive quality (Lee et al. 2021).

Benefits of intercropping include the capacity to reduce N fertilizer input by increasing N use-efficiency. This is in part because N loss to the environment is a major pollution source through N leaching, runoff, or emissions (Hendricks et al., 2019; Liang et al., 2021; Qiang et al., 2020). Preventing N overfertilization and optimizing N fertilizer usage can be pursued by accounting for the underlying soil moisture availability (Thilakarathna et al., 2020) as well as by choosing an appropriate N fertilizer rates (Chai et al., 2020). Additionally, N fertilizer rates need to account for any interaction effects between plant species within a given intercropping system (e.g., contribution of legume crops to overall N availability).

While most of the effects of intercropping are attributed to interactions between plant species, there are studies demonstrating that certain plant-microbial interactions are also involved;

specifically the interaction between plants and soil mycorrhizal fungi (Koide 1991, Li et al. 2014). There is also evidence from multi-species fields that plants can share nutritional resources via transport channels created by mycorrhizal symbiosis (Hauggaard-Nielsen and Jensen 2005, Bever et al. 2009). Where plants acquire or exchange more nutrients through these mycorrhizal channels, they could require less exogenous fertilizers.

There are many studies that focus on the role of arbuscular mycorrhizal fungi (AMF) between perennial crops growing together (Egerton-Warburton et al. 2007, Gollotte et al. 2004, Johnson 1993, Miller et al. 2002), and fewer on annual crop species (Correa et al. 2014, Ryan and Angus 2003), and seldom in intercropping systems (Hauggaard-Nielsen and Jensen 2005). Relative to oat and pea monocrops, this study examines how oat-pea intercropping with and without N fertilizer addition affects AMF richness and diversity, including both the bulk soil and root compartments. In line with previous studies on perennial plant species, we anticipate that the AMF root community to change as a response to intercropping and N fertilizer addition.

4.3 Methods

4.3.1 Site Description and Experimental Design

Field experiments were carried out over two growing seasons – from 30 May to 30 Aug. 2019, and from 22 Jun. to 21 Sep. 2020, at the Fairview Research Farm, located in north-western Alberta, Canada (56°04'53.3"N, 118°26'25.1"W). The study was conducted using new plots each year. This region experiences long and cold winters and short and mild summers, and is characterized as a boreal climate. The soil at the experimental site is classified as an Eluviated Black Chernozem, according to the Canadian System of Soil Classification (Agricultural Region of Alberta Soil Inventory Database AGRASID; Government of Alberta, 2020). The management history of the experimental sites was a long-term alfalfa stand for hay production until termination with Roundup WeatherMax® herbicide (glyphosate) the fall of 2018 (September) before the experiments commenced (Lee et al., 2021). The long-term temperature and rainfall averages, as well as the observed temperature and rainfall for 2019 and 2020 were acquired through the Alberta Climate Information System (ACIS 2020) from a permanent weather station located on site (Table 1). Baseline soil sample collection and analyses were done before crop seeding in both years. Soil availabilities of nitrogen (N) and phosphorus (P) were deficient. Upon alfalfa stand termination in fall 2018, the field was deep plowed, disced and harrowed. The sites were lightly harrowed before seeding operations.

The two plant species in this experiment were oats (*Avena sativa* L., O), peas (*Pisum sativum* L., P), and were intercropped as oat-pea (OP). Seeding rates had the plant density targets of 300 plants m^{-2} for monocrop oats and 90 plants m^{-2} for monocrop peas. Within the 2-species intercropping, each plant species was seeded at 75% of their common seeding rates.

A factorial randomized complete block design was implemented, where the two factors were intercropping systems (O, P, OP) and with or without N fertilizer (fertilizer rate was applied at 75 kg N ha^{-1}) with four replicates. On 30 May, 2019 and 22 Jun., 2020, we used a 6-row small-plot seeder (equipped with disc-type openers) with a 22.9 cm row spacing to seed into 2 m by 16 m plots, and the base fertilizer (a mixture of P, K, and S, Table 1) and N fertilizer addition (granular urea, 46-0-0) were banded at seeding. To reduce weed competition, we applied glyphosate (StartUp® Roundup) herbicide as pre-emergent in 2019 and pre-pass in 2020 as well as occasional hand weeding.

4.3.2 Field Sample Collection

For mycorrhizal analytical samples, three locations within each experimental plot were sampled using a sterile trenching shovel, and individually separated into three bulk soil samples and three plant root samples. The plant root samples were then subsampled for microscopy and molecular analysis, where the molecular samples were stored at -20°C while the microscopy samples were stored in ethanol until ready for analysis. Bulk soil samples were not subsampled and were also stored at -20°C until ready for analysis.

Experimental data of forage yield (biomass productivity), plant water use-efficiency (WUE) and forage nutritional quality at the same field plots were taken from a study on intercropping productivity as an alternative forage source to conventional cattle feed (Lee et al. 2021).

4.3.3 Root colonization

Root samples were washed with distilled water until free of soil. 0.1g of root sample is collected and stained using the procedure detailed in (McGonigle et al. 1990), where roots were cleared in 10% KOH and stained with Trypan blue solution for 30min, stored on slides at 4C until examined. The roots are then placed on a compound microscope and counted for hyphae, arbuscules, and vesicles per root intersection. Each part is recorded as a ratio of hyphae/arbuscule/vesicle count: intersection.

4.3.4 Molecular analysis

Total DNA was extracted from 0.25 g of samples using the PowerSoil DNA Isolation kit according to the manufacturers' guideline (MoBio Laboratories Inc., Carlsbad, California). DNA purity and concentration were measured by a Biodrop spectrophotometer (Biochrom, Cambridge, UK) and a Qubitv4 fluorimeter using a Qubit™ dsDNA BR Assay Kit (ThermoFisher Scientific, Massachusetts, USA), respectively. The amplificability of the AMF small subunit (SSU) region were confirmed with a polymerase chain reaction (PCR) test by using primer pair NS31 (5'-TTGGAGGGCAAGTCTGGTGCC-3') to AML2 (5'-GAACCCAAACACTTTGGTTTCC-3'). The PCR cycling conditions were: 94 ºC (3 min); 35 cycles of 94 ºC (45 s), 63 ºC (60 s), and 72 ºC (90 s); followed by final extension step 72 ºC (10 min) (Morgan et al., 2017). The fragment size and quality of amplification of the PCR product was verified by electrophoresis on 1% agarose gel.

Sequencing was implemented using an Illumina MiSeq PE300 platform at a read length of 2 × 300 bp (Illumina Inc., San Diego, California, USA). Using the same primer pair as the set used in PCR test, the metagenomics data was extracted from the samples. The metagenomics data was then processed further using the Fluidigm sequencing adaptor to produce a genetic library. The library prep was completed by Genome Quebec (Quebec, Canada).

4.3.5 Bioinformatics and statistical analysis

The raw FASTQ data was processed with the Qiime2 pipeline (version 2019.10 https://qiime2.org/) (Bolyen et al., 2019). DADA2 algorithm was used to implement error correction, quality filtering, chimera removal and sequence variance of Illumina amplicon sequences (Callanhan et al., 2016). The first 21 bp and 22 bp in the forward and reverse reads were trimmed for removing primers, respectively. The forward and reverse reads were truncated at 295 and 283 bp, corresponding to average quality score (Phred Q score) of higher than 20, respectively. These quality criteria encompassing denoised, merged, and non-chimeric yields a loss of 73.5 % to whole sequence reads. Thereafter, amplicon sequence variants (ASVs) were clustered with ≥ 97% similarity in an open-reference picking process using classify-consensus-vsearch in Qiime2. Clustered ASVs (hereafter, referred as operational taxonomic units; OTUs) for taxonomic identification was retrieved from directly querying the MaarjAM database (Őpik et al., 2010). Unassigned OTUs were further aligned against a Silva 138 99% OTUs sequences reference database. Among the blasted taxonomy against two database, non-Glomeromycotina fungi for the 18S sequences were removed from subsequent analyses to constrain analysis to the target groups. Singletons and OTUs present in less than three samples were removed from the analysis. The processed data were exported from Qiime2 to analyze and visualize within the R packages ‘phyloseq’ and ‘vegan’ (Oskanen et al. 2020, Fukuyama 2020).

Alpha diversity of AMF communities was evaluated by Chao1 richness, Pielou’s evenness, Shannon’s diversity, and inverse Simpson’s diversity indices with a linear mixed model as the parametric test. The linear mixed model was used for analysis of variance (ANOVA) of AMF colonization as well as ANOVA of alpha diversity. By doing so, a random effect (‘block’) was removed while statistically analyzing crop and fertilizer effect on colonization and alpha diversity using the R package. Normality and homoscedasticity of the model residuals were assessed using Shapiro-Wilk and function, respectively (NLME package of 3.2.1; R Core Team, 2015). Box-Cox transformation were applied to correct non-normality or heteroscedasticity when needed. Pairwise comparisons were conducted after significant ANOVA with Tukey’s Honest Significant Difference (HSD) using agricolae package in R (de Mendiburu and Felipe, 2020).

Before statistical analysis of beta diversity, OTUs absolute count data were transformed for even sampling depth based on ‘phyloseq’ tutorial (McMurdie and Holmes, 2013). The beta significance of AMF communities was assessed by permutational multivariate analysis of variance (PERMANOVA) and illustrated by non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrices (Anderson et al., 2018). The differential abundance test was performed by edgeR: a Bioconductor package (Robinson et al., 2010). Transformation-based canonical correspondence analysis (tb-CCA) and redundancy analysis (tb-RDA) were used to explain dissimilarity with environmental variables. Spearman correlation test was performed to find relationships between dominant taxa and environmental variables.

4.4 Results

4.4.1 Alpha diversity

We found differences in AMF species richness estimates in the roots across treatment combinations (i.e., intercropping systems x N fertilizer rate) in 2019 (Chao1, $p < 0.05$, Table 2). In oat, a decrease in AMF richness occurred in soils receiving N fertilizer, while an increase in AMF richness occurred in Pea with the N fertilizer application (Fig. 1B, Chao1). The richness of AMF species remained relatively similar in oat-pea irrespective of N fertilizer addition (Fig. 1B, Chao1). No significant differences in species richness were observed in 2020. Instead, we observed a difference in AMF species diversity in the roots across the cropping systems, but not in 2019 (Shannon, Table 2). Pea and oat-pea had the highest AMF diversity, while oat had the lowest (Fig. 3B, Shannon).

4.4.2 Community composition

The NMDS plots showed spread in root AMF communities between cropping options (Fig. 2A, 2B). In statistical analysis, PERMANOVA also exhibited a significance of AMF community composition within roots; furthermore, the pairwise community composition comparison indicated that the AMF communities were significantly different across each of the three cropping systems ($Ps < 0.05$; Table 3, Table 4).

The evident differences in AMF community composition between the two study years in the bulk soil were significantly associated with forage nutritive indicators across all experimental units (Supplementary Fig 1A), specifically K, Na, and P concentrations in harvested forages ($Ps < 0.05$, model $P < 0.001$). In the root compartment, the AMF community composition between the two study years were also significantly associated with forage nutritive indicators (Supplementary Fig 1B), specifically neutral detergent fibre (NDF), calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) concentrations, biomass productivity as well as water use-efficiency (WUE) (Ps < 0.05, model $P < 0.001$). In further details, the differences in AMF community composition across cropping systems in 2019 were significantly associated with forage indicators (Supplementary Fig 1C), specifically biomass, crude protein, NDF, WUE, and most mineral contents (Ca, K, Mg, Na) in the harvested forage (Ps < 0.05, model $P < 0.001$). In 2020, while the differences in AMF community composition between cropping systems were significantly associated with forage nutritive indicators (model $P < 0.01$), the fitted vectors did not significantly correlate with any of the AMF community composition of each cropping system.

Besides a significant difference between cropping systems, a significant difference in AMF communities was also observed between the roots versus soil compartment (Table 4). AMF alpha diversity between the two compartments was significantly different in both years (Table 5). Not only was there a difference in richness of AMF species (Chao1, $P < 0.05$, Table 5), but there was also a difference in species diversity (Evenness, Shannon, InvSimpson, $Ps < 0.05$, Table 5). The NMDS plots indicated that there was no overlap in AMF community between the roots and soil compartment (Fig. 2C, Fig. 2D). Concomitantly, PERMANOVA also showed a significant difference between two compartments (Table 4). Noticeably, this effect was observed when we pooled the AMF communities across all experimental samples; the similarities were greater between compartments (roots vs. bulk soil) rather than between years (2019 vs. 2020) (Fig. 4).

The soil community alpha diversity did not differ significantly (Table 2). We observed overlapped communities when comparing between intercropping systems (Fig. 5A, Fig. 5B), as well as in N fertilizer rates (Fig. 5C, Fig. 5D, Fig. 5E, Fig. 5F).

Diversispora richness in 2019 was negatively correlated with both biomass production and WUE, while positively correlated to crude protein content in the forage ($Ps < 0.05$, Supplementary Fig 2A). Conversely, crude protein was negatively correlated with *Paraglomus* in 2019, compared to *Diversispora* ($P < 0.05$). In 2020, *Diversispora* richness was positively correlated with forage Mg concentration ($P < 0.05$, Supplementary Fig 2A). There were significant correlations with specific mycorrhizal genera in the roots in 2019 ($Ps < 0.05$, Supplementary Fig 2B); *Diversispora* richness was negatively correlated with forage biomass production while positively correlated with forage Ca and crude protein contents ($Ps < 0.01$), and *Paraglomus* richness was negatively correlated with Ca while *Claroideoglomus* richness was positively correlated with Ca ($Ps < 0.05$). In the same year, NDF and WUE were positively correlated with *Paraglomus* richness, but negatively correlated with *Diversispora* and *Claroideoglomus* ($Ps < 0.05$, Supplementary Fig 2B). No significant correlations in both soil and root compartments could be found in 2020 (Supplementary Fig 2C).

In terms of relative abundance, 80.14% of the AMF community was assigned to phylum Glomeromycota in 2019, and 67.74% in 2020 (Fig. 6). Differential abundance analysis was implemented to see how cropping systems could influence specific genus. There were significant treatment impacts observed within the soil and root compartments ($Ps < 0.05$; Table 6, Table 7, Table 8, and Table 9), as well as significant differences when comparing the bulk soil vs. root compartments ($Ps < 0.05$; Table 10). However, there was no significant effects of N fertilizer addition on AMF community within the soil or root compartments (Table 6, Table 7, Table 8, and Table 9).

In the roots compartment in 2019, a proportional difference was observed in genera *Claroideoglomus* and *Diversispora* between oat and pea ($p < 0.01$, Table 9). There was significantly higher content of *Claroideoglomus* and *Diversispora* in pea compared to oat (Fig. 7B). In the same year, between pea and oat-pea, a proportional difference was observed in *Paraglomus* (Table 7). There was significantly higher content of *Paraglomus* in oat-pea compared to pea (Fig. 7B). In the soil compartment in 2020, a propotional difference was observed in *Archaeospora* between pea and oat-pea (P<0.01, Table 7). There was a significant higher presence of *Archaeospora* in oat-pea compared to pea alone (Fig. 7C). In the roots compartment in 2020, *Diversispora* abundance was higher in pea compared to oat, while *Paraglomus* abundance was highest in oat (*Ps* <0.05; Table 9, Fig. 7D). In terms of compartmental differences (roots vs. bulk soil), *Ambispora* and *Archaeospora* were found to be significantly different in both years, *Claroideoglomus* and Glomus in 2019, and *Paraglomus* in 2020 (Table 10). In both years, there was a higher abundance of *Acaulospora* and Archaeospora in the bulk soil compartment compared to the roots compartment (*Ps* <0.05; Table 10, Fig. 8). There was a higher abundance of *Claroideoglomus* and Glomus in the root compartment in 2019 (*Ps* <0.05; Table 10, Fig. 8A), and there was a higher abundance of *Paraglomus* in the root compartment in 2020 (*P* < 0.05; Table 10, Fig. 8B).

4.4.3 Root colonization

In the microscopy of hyphae, arbuscule, and vesicle infection in roots, we did not observe any interactions between cropping systems and N fertilizer addition (data not shown). Likewise, there were no significant effects of cropping systems or N fertilizer addition.

4.5 Discussion

In this study, we found that field management factors including cropping choice and N fertilizer application had significant impacts on AMF diversity. We also show that, as previously demonstrated (Chen et al. 2018, Philpott et al. 2013), root and soil compartments host different

AMF community composition. Previous studies have also shown that field management impacts AMF communities (Broeckling et al. 2008, Chen et al. 2018).

We observed higher alpha diversity index values (Chao1 in 2019, Fig. 1A; Shannon in 2020, Fig. 3B) in oat-pea intercropping than either monocropped oat or pea. Since the diversity increase in intercropping oat with pea does not equal the sum of the diversity of oat alone and pea alone, we infer that the mycorrhizal communities overlap between oat and pea, as well as syngergy supporting a higher diversity of AMF when two functionally complimentary plant species are grown together. Incombining the two crop species, the root exudates may help to recruit and maintain an environment suitable for mycorrhizal species commonly present in both plant species (Broeckling et al. 2008).

In 2019, where the rainfall was below normal average (Table 1), AMF richness was affected by both cropping choice and N fertilizer addition (Table 2). With oat and oat-pea, a decrease in AMF richness occurred when adding N fertilizer into the system. Conversely, pea experienced the exact opposite effect whereby adding N fertilizer increased AMF richness. In previous studies, N fertilization altered mycorrhizal communities (Egerton-Warburton 2007, Johnson 1993). Johnson (1993) suggested that the addition of N fertilizer causes plants to select for inferior species of mycorrhizae. With an increase in forage biomass production as a response to fertilizer addition in oat (Lee et al. 2021), there was a decrease in AMF richness (Fig. 1B). With this, one hypothesis is that oat, like perennial grasses, benefit from the N fertilizer addition, and thus would benefit less from mycorrhizal symbiosis (Johnson et al. 2008). This would lead to less recruitment, as mycorrhizal infection would deem to be an inefficient growing strategy. Since peas do not benefit from adding N fertilizer as this reduces rhizobium activity (Clayton et al. 2003, Huang et al. 2017), the recruitment of mycorrhiza becomes the more effective strategy for nutrient acquisition in pea fields receiving N fertilizer additions. As a result, intercropping oat and pea can be conceptualized as an effective strategy in managing the mycorrhizal community as oat and pea have different requirements of N fertilizer additions, which was reflected in how the relative abundance of mycorrhizal species remained similar when comparing with and without N fertilizer addition (Fig. 1A).

From observing differences between the root and soil samples, as well as between the treatments, we conclude that plant preferences determine the root mycorrhizal community. The soil mycorrhizal community, which was generally more diverse than the root community (Table 5), serves as the inoculum pool that living root selects from and represents the spores deposited by plants historically present (Broeckling et al. 2008). As a result, studies have shown that monocropping, fungicide use, and synthetic nutrient application can reduce the inoculum pool, which limits the optimization of the mycorrhizal synergy (Nelson and Spaner 2010, Johnson 1993, Ying et al. 2018). The lack of plant diversity decreases the introduced spores into the system, and without the plant-fungal symbiosis due to synthetic nutrient dependency, the mycorrhizae are less likely to survive.

Within the observed variety of AMF genera in the roots, *Diversispora* was significantly more abundant in pea than in oats in each of the two growing seasons. Additionally, while no significant difference was found between oat and oat-pea, oat-pea does consistently have higher abundance of *Diversispora* than oat (Table 8, Table 9). Curiously, *Clarideoglomus* was greatly recruited only by peas in 2019 under drier soil conditions (Fig. 7B), and not in 2020 (Fig. 7D). This phenomenon of peas exclusively recruiting *Clarideoglomus* was seen in both the bulk soil and roots compartments (Fig. 8). Collectively, this finding can suggest that this AMF genus could be moisture sensitive. We thus hypothesize that the genus *Diversispora* is a key group of mycorrhizae that peasdepend on more consistently, while that the genus *Clarideoglomus* is facultatively recruited depending upon the prevalence of dry climate conditions. However, there lacks research detailing the roles and functions of this genus, hence this is a topic that requires further exploration.

Similarly, we witnessed that *Paraglomus* was significantly higher in oat-pea than in pea in the drier 2019, while significantly lower in oat-pea than in oat in the wetter 2020. This relationship could reveal which plant species within the intercropping system the mycorrhizal community is prioritizing to associate with as a function of underlying soil moisture conditions. This is inferred because the *Paraglomus* abundance in oat-pea was between oat and pea; however, it was more similar to one of the two monocrops depending on experiencing high or low rainfall during a given growing season. As peas can access deeper water reservoirs due to their taproot system, they would tend to grow better in drier environments compared to oats, which would have a shallower fibrous root system. Conversely, a taproot system would not be able to advantageously capitalize on moisture resources in a wetter environment. To elaborate further, this finding may be an indication that pea exudation naturally deters the presence of *Paraglomus*, which could imply that this AMF genus might be harmful or not beneficial for pea growth. As plant stress is a factor that increases exudation (Karst et al. 2017, Nian et al. 2002), this result demonstrates that within an intercropped field, the stressed plants can persist by accessing soil resources via the fungal network, while the dominant plant species without stress simply capitalizes directly on the favorable conditions. While this finding does not refute the notion that mycorrhizae have a transport channel that shares nutrients among plants (both intra-species and inter-species) (Hauggaard-Nielsen and Jensen 2005, Bever et al. 2009), it does pose the question as to whether mycorrhizae simply share the nutrients gathered from the soil solution through the fungal network rather than transferring nutrients directly from one plant to another within a field.

4.6 Conclusion

This study enforces that AMF communities are highly controlled by plant-soil interactions, specifically how different plant species and growing conditions play a role in the structuring of mycorrhizal communities. This study also demonstrated that mycorrhizal species richness increases with intercropping likely because root exudation from multiple plant species in intercropping can recruit mycorrhizae and create an overlapped AMF community that collectively benefits the intercropped plant species. With N fertilization, we noticed a decrease in richness in AMF species. Furthermore, we ascertained two AMF genera indicators: i) *Diversispora* presence indicated good growing environments for peas, and ii) *Paraglomus* was linked to a stressed plant species within the oat-pea intercropping system.

Overall, this study prompts further research to focus on the optimal N fertilizer addition to balance between adequate yield gain and AMF species retention in the soil, as well as the role of *Diversispora* and *Paraglomus* in oat and peas monocrops and intercropping. Specifically, why these AMF genera need to be present or absent to underpin the growth of annual crop species planted alone or in combination.

4.7 References

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4.8 Tables and Figures

Table 4.1. Rainfall, temperature, and soil properties (0-15 cm depth increment) at the experimental site, as well as the basal fertilizer applied in 2019 and 2020[9]

	2019	2020	Long Term Average
Month	Rainfall (mm)		
June	72.9	67.2	64.5
July	61.9	89.8	69.5
August	49.1	53.9	47.5
September	24.6	23.1	33.7
Total	208.5	234.0	215.2
	Air temperature (°C)		
June	14.1	14.0	14.0
July	15.1	15.6	15.9
August	12.9	14.1	14.6
September	9.7	10.3	9.6
Soil property			
Nitrate-N (mg kg^{-1})	5	24	
P (mg kg^{-1})	7	10	
K (mg kg^{-1})	242	216	
S (mg kg^{-1})	5	5	
Ca (mg kg^{-1})	1920	1760	
Mg (mg kg^{-1})	354	359	
pH	6.2	6.2	
Organic matter, OM (%)	8.2	3.7	
Fertilizer Blend Applied			
P_2O_5 (kg ha^{-1})	27.2	10.9	
K_2O (kg ha^{-1})	27.2	Nil*	
S (kg ha^{-1})	12.7	13.6	

* Soil tests indicated adequate availability of soil K, so no additional K_2O was applied.

Table 4.2. Alpha diversity in bulk soil and roots samples across the crop × N fertilizer effects with two-way ANOVA in 2019 and 2020.[10]

		Soil		Roots	
		2019			
Parameter	Treatment	F-value	P-value	F-value	P-value
Chao1	Crop	0.1277	0.8809	1.5972	0.2350
	Fertilizer	0.0286	0.8677	0.0000	1.0000
	Crop: Fertilizer	1.8561	0.1904	6.2173	<0.05*
Evenness	Crop	0.127	0.8809	1.1698	0.3342
	Fertilizer	0.0286	0.8677	0.1282	0.7247
	Crop: Fertilizer	0.6214	0.5505	0.5653	0.5799
Shannon	Crop	0.1277	0.8809	1.7151	0.2097
	Fertilizer	0.0286	0.8677	0.1706	0.6847
	Crop: Fertilizer	0.1875	0.8310	1.8105	0.1975
InvSimpson	Crop	0.1277	0.8809	1.3639	0.2822
	Fertilizer	0.0286	0.8677	0.2454	0.6267
	Crop: Fertilizer	0.1460	0.8653	1.9728	0.1735
		2020			
Chao1	Crop	0.7225	0.4999	0.6277	0.5457
	Fertilizer	0.0069	0.9348	0.8229	0.3770
	Crop: Fertilizer	0.9727	0.4007	0.7605	0.4846
Evenness	Crop	0.400	0.6762	2.9941	0.0769
	Fertilizer	1.839	0.1928	2.9000	0.1068
	Crop: Fertilizer	0.432	0.6573	0.0587	0.9432
Shannon	Crop	0.0366	0.9641	4.9846	<0.05*
	Fertilizer	1.3474	0.2618	1.0715	0.3151
	Crop: Fertilizer	1.264	0.3110	0.2116	0.8166
InvSimpson	Crop	0.0218	0.9785	2.90686	0.0821
	Fertilizer	0.9228	0.3502	0.40540	0.5328
	Crop: Fertilizer	0.7504	0.4891	0.50203	0.6151

Number of degree freedom (NumDF) is [Crop = 2, Fertilizer = 1, Crop: Fertilizer = 2]. Denominator of Degree of freedom (denDF) is 15 and 17, in soil and roots, respectively.

Table 4.3. Pairwise community composition comparison between cropping options in root samples based on PERMANOVA. P value adjusted by false discovery rate (FDR). [11]

	Oat	Pea
	*2019-Roots (F=15.27, P<0.001***)*	
Pea	0.0015	-
Oat-Pea	0.0020	0.0015
	*2020-Roots (F=7.01, P<0.001***)*	
Pea	0.003	
Oat-Pea	0.005	0.003

Significance codes: P<0.05, *; P<0.01, **; P<0.001, ***.

Table 4.4. PERMANOVA across combination cropping systems (crop × N fertilizer) and root compartments (bulk soil vs. roots).[12]

		2019			2020	
Treatment	F.model	R^2	Pr(>F)	F.model	R^2	Pr(>F)
			Bulk soil			
Crop	0.728	0.067	0.743	1.012	0.092	0.276
Fertilizer	0.658	0.030	0.762	0.640	0.029	0.802
Crop : Fertilizer	0.810	0.074	0.639	0.635	0.0579	0.923
			Roots			
Crop	14.92	0.592	<0.001***	6.483	0.400	<0.001***
Fertilizer	0.254	0.005	0.906	0.524	0.016	0.698
Crop : Fertilizer	1.134	0.045	0.379	0.451	0.027	0.878
			Rhizosphere			
Compartment	39.179	0.460	<0.001***	16.241	0.261	<0.001***

Significance codes: $P<0.05$, *; $P<0.01$, **; $P<0.001$, ***.

Table 4.5. AMF alpha diversity. Average and standard errors (n=24) of soil and root samples from 2019 and 2020 across treatments. Metrics include Chao1 richness, Peilou's evenness, Shannon's diversity, and inverse Simpson's diversity. Italics indicate the P-value.[13]

Treatment	Chao1	Evenness	Shannon	InvSimpson
	2019			
Bulk soil	24.12±1.08a¶	0.67±0.02b	2.10±0.06b	5.49±0.38b
Roots	26.81±0.86b	0.56±0.02a	1.83±0.05a	4.42±0.28a
	*<0.05**	*<0.001****	*<0.001****	*<0.01***
	2020			
Bulk soil	39.10±1.07b	0.75±0.01b	2.76±0.04b	10.62±0.50b
Roots	32.80±1.06a	0.59±0.01a	2.05±0.05a	5.31. ±0.34a
	*<0.001****	*<0.001****	*<0.001****	*<0.001****

¶Pairwise comparisons were performed with Tukey's HSD test after ANOVA.

Lowercase letters signify significant difference between treatments based on Tukey HSD test after ANOVA

Table 4.6. Differential abundance in genus rank impacted by treatments (crop × N fertilizer) in soil compartment in 2019.[14]

Genus	logFC	logCPM	LR	P-value	FDR
	Crop				
	Oat vs. Pea				
Acaulospora	0.0000	6.6919	0.0000	1.0000	1.0000
Ambispora	-0.1827	14.4043	0.0593	0.8076	1.0000
Archaeospora	0.3378	16.6234	0.5555	0.4561	1.0000
Claroideoglomus	-0.1858	16.0487	0.0503	0.8225	1.0000
Diversispora	0.3296	12.8606	0.0267	0.8703	1.0000
Geosiphon	6.7054	9.1622	0.7148	0.3979	1.0000
Glomus	0.3810	16.7911	0.9856	0.3208	1.0000
Paraglomus	-0.1210	19.3964	0.8122	0.3675	1.0000
Scutellospora	0.2404	12.7747	0.0051	0.9432	1.0000
	Oat vs. Oat-Pea				
Acaulospora	0.0000	6.9199	0.0000	1.0000	1.0000
Ambispora	-0.4891	14.2243	0.4162	0.5189	0.7542
Archaeospora	-0.2405	16.2658	0.3292	0.5661	0.7542
Claroideoglomus	-0.4853	15.7479	0.4994	0.4798	0.7542
Diversispora	1.1069	13.2736	0.4184	0.5177	0.7542
Geosiphon	0.0000	6.9199	0.0000	1.0000	1.0000
Glomus	0.8955	17.0283	7.5819	0.0059	0.0531
Paraglomus	0.1025	19.4419	0.5779	0.4471	0.7542
Scutellospora	-2.3916	11.2163	0.2957	0.5866	0.7542
	Pea vs. Oat-Pea				
Acaulospora	0.0000	6.6198	0.0000	1.0000	1.0000
Ambispora	-0.5033	14.1489	0.5275	0.4676	0.7173
Archaeospora	-0.4822	16.3873	2.5040	0.1136	0.5110
Claroideoglomus	-0.2404	15.6921	0.2484	0.6182	0.7782
Diversispora	0.5859	13.3669	0.1573	0.6917	0.7782
Geosiphon	-6.4775	8.9165	0.6698	0.4131	0.7173
Glomus	0.4702	17.1270	2.8194	0.0931	0.5110
Paraglomus	0.1491	19.4316	0.5030	0.4782	0.7173
Scutellospora	-2.6625	13.1324	1.8339	0.1757	0.5270
	N Fertilizer				
Acaulospora	0.0000	6.7347	0.0000	1.0000	1.0000
Ambispora	0.5864	14.2646	1.1714	0.2791	0.5243
Archaeospora	-0.0989	16.4048	0.1031	0.7482	0.9094
Claroideoglomus	-1.0752	15.8327	4.2421	0.0394	0.1774
Diversispora	1.4918	13.2102	1.1136	0.2913	0.5243
Geosiphon	5.9028	8.5833	0.6269	0.4285	0.6427
Glomus	0.0711	17.0027	0.0588	0.8084	0.9094
Paraglomus	-0.1983	19.4435	1.4910	0.2221	0.5243
Scutellospora	4.4595	12.8212	5.0883	0.0241	0.1774

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at *P* <0.05, *P* <0.01, *P* <0.001, respectively.

Table 4.7. Differential abundance in genus rank impacted by treatments (crop × N fertilizer × compartment) in soil compartment in 2020.[15]

Genus	logFC	logCPM	LR	P-value	FDR
	Crop				
	Oat vs. Pea				
Acaulospora	-0.8035	7.9600	0.0243	0.8762	0.8762
Ambispora	1.3911	12.7442	1.9456	0.1631	0.3669
Archaeospora	-0.4631	16.8018	2.3644	0.1241	0.3669
Claroideoglomus	-0.0751	16.4194	0.0321	0.8577	0.8762
Diversispora	1.0663	14.6686	2.2826	0.1308	0.3669
Geosiphon	-1.2063	8.8371	0.0833	0.7728	0.8762
Glomus	-0.2243	18.6496	1.1247	0.2889	0.4334
Paraglomus	0.2100	18.4620	1.2367	0.2661	0.4334
Scutellospora	-5.2554	12.6132	4.3738	0.0365	0.3285
	Oat vs. Oat-Pea				
Acaulospora	-2.0251	7.8935	0.1519	0.6967	0.8405
Ambispora	1.1497	12.5062	0.7125	0.3986	0.7175
Archaeospora	0.6636	17.3084	4.1327	0.0421	0.3174
Claroideoglomus	0.8312	16.7974	3.2705	0.0705	0.3174
Diversispora	1.2925	14.4156	1.6542	0.1984	0.5188
Geosiphon	0.2545	9.7585	0.0069	0.9337	0.9337
Glomus	-0.2533	18.4851	1.4373	0.2306	0.5188
Paraglomus	0.0762	18.3246	0.1040	0.7471	0.8405
Scutellospora	-2.5044	13.1511	0.2928	0.5884	0.8405
	Pea vs. Oat-Pea				
Acaulospora	-1.5681	7.3397	0.1979	0.6564	0.8440
Ambispora	-0.3632	13.0798	0.2636	0.6077	0.8440
Archaeospora	1.0840	17.1833	13.7664	0.0002	P<0.01**
Claroideoglomus	0.7903	16.7608	5.0433	0.0247	0.1112
Diversispora	0.1498	14.8059	0.0423	0.8371	0.9295
Geosiphon	0.9734	9.5768	0.0078	0.9295	0.9295
Glomus	-0.2126	18.4218	1.7160	0.1902	0.5706
Paraglomus	-0.1168	18.4252	0.5477	0.4593	0.8440
Scutellospora	2.3645	11.5246	0.4666	0.4945	0.8440
	N Fertilizer				
Acaulospora	-0.7035	7.7010	0.0242	0.8765	0.9273
Ambispora	0.3361	12.7365	0.2220	0.6376	0.8490
Archaeospora	-0.2095	17.1126	0.5014	0.4789	0.8490
Claroideoglomus	-0.3184	16.6616	0.8260	0.3634	0.8177
Diversispora	-0.6853	14.5830	1.1800	0.2773	0.8177
Geosiphon	0.4407	9.3635	0.0083	0.9273	0.9273
Glomus	-0.2528	18.5733	1.6210	0.2030	0.8177
Paraglomus	-0.1462	18.3868	1.0832	0.2980	0.8177
Scutellospora	1.4579	12.4333	0.1931	0.6603	0.8490

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

Table 4.8. Differential abundance in genus rank impacted by treatments (crop × N fertilizer) roots compartment in 2019.[16]

Genus	logFC	logCPM	LR	P-value	FDR
	Crop				
	Oat vs. Pea				
Acaulospora	0.0000	5.4251	0.0000	1.0000	1.0000
Ambispora	0.4397	9.4917	0.0613	0.8044	1.0000
Archaeospora	1.4919	14.1683	3.4482	0.0633	0.1425
Claroideoglomus	1.9763	16.8508	11.0531	0.0009	<0.01**
Diversispora	4.2749	14.5509	10.4065	0.0013	<0.01**
Geosiphon	0.0000	5.4251	0.0000	1.0000	1.0000
Glomus	0.4968	18.1753	1.1880	0.2757	0.4963
Paraglomus	-0.6351	19.1178	3.9704	0.0463	0.1389
Scutellospora	-0.1931	10.9673	0.0065	0.9355	1.0000
	Oat vs. Oat-Pea				
Acaulospora	0.0000	5.5051	0.0000	1.0000	1.0000
Ambispora	-0.1349	9.3792	0.0060	0.9382	1.0000
Archaeospora	0.1549	13.2765	0.0532	0.8176	1.0000
Claroideoglomus	0.7652	15.9601	2.5889	0.1076	0.4843
Diversispora	1.0324	11.7888	0.4610	0.4972	1.0000
Geosiphon	0.0000	5.5051	0.0000	1.0000	1.0000
Glomus	0.0422	17.9462	0.0084	0.9271	1.0000
Paraglomus	-0.1901	19.4004	0.5745	0.4485	1.0000
Scutellospora	-5.6064	8.8917	3.6738	0.0553	0.4843
	Pea vs. Oat-Pea				
Acaulospora	0.0000	5.3631	0.0000	1.0000	1.0000
Ambispora	-0.4987	9.6272	0.0665	0.7965	1.0000
Archaeospora	-0.8562	14.0865	1.2727	0.2593	0.3889
Claroideoglomus	-0.7357	16.9400	2.4024	0.1212	0.2726
Diversispora	-2.2442	14.4101	3.7358	0.0533	0.1728
Geosiphon	0.0000	5.3631	0.0000	1.0000	1.0000
Glomus	-0.5461	18.1151	1.6018	0.2056	0.3702
Paraglomus	1.0818	19.2547	8.0149	0.0046	<0.05*
Scutellospora	-5.0267	11.6952	3.6051	0.0576	0.1728
	N Fertilizer				
Acaulospora	0.0000	5.4648	0.0000	1.0000	1.0000
Ambispora	-0.6643	9.4827	0.1700	0.6801	1.0000
Archaeospora	0.4050	14.0053	0.3659	0.5452	1.0000
Claroideoglomus	-0.3611	16.7550	0.4797	0.4885	1.0000
Diversispora	-1.4589	14.1711	1.5484	0.2134	1.0000
Geosiphon	0.0000	5.4648	0.0000	1.0000	1.0000
Glomus	0.1082	18.1213	0.0695	0.7920	1.0000
Paraglomus	-0.2918	19.1779	1.3498	0.2453	1.0000
Scutellospora	-0.0588	10.4827	0.0010	0.9742	1.0000

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

Table 4.9. Differential abundance in genus rank impacted by treatments (crop × N fertilizer × compartment) in the roots compartment in 2020.[17]

Genus	logFC	logCPM	LR	P-value	FDR
	Crop				
	Oat vs. Pea				
Acaulospora	1.3400	5.7791	0.4181	0.5179	0.7769
Ambispora	0.3758	8.8405	0.0480	0.8265	0.8265
Archaeospora	0.2148	15.4236	0.1393	0.7090	0.8265
Claroideoglomus	0.1373	16.3185	0.0664	0.7966	0.8265
Diversispora	4.9308	13.8508	14.1982	0.0002	<0.001***
Geosiphon	-2.9298	7.1943	1.1140	0.2912	0.5242
Glomus	0.9078	18.6071	5.7419	0.0166	0.0497
Paraglomus	-0.9956	18.8468	15.3313	0.0001	<0.001***
Scutellospora	-4.1443	10.5788	1.7651	0.1840	0.4140
	Oat vs. Oat-Pea				
Acaulospora	0.0000	5.7105	0.0000	1.0000	1.0000
Ambispora	0.7853	9.1257	0.2531	0.6149	0.9731
Archaeospora	0.0805	15.7239	0.0187	0.8912	1.0000
Claroideoglomus	0.2639	16.5379	0.2075	0.6487	0.9731
Diversispora	2.9417	12.3120	2.2484	0.1338	0.6019
Geosiphon	-2.9781	7.3468	1.0293	0.3103	0.9310
Glomus	-0.3128	18.1497	0.4128	0.5206	0.9731
Paraglomus	-0.9081	19.1818	9.3501	0.0022	<0.05*
Scutellospora	-1.7704	11.4713	0.0845	0.7712	0.9916
	Pea vs. Oat-Pea				
Acaulospora	-1.2608	5.7162	0.4546	0.5002	0.6431
Ambispora	0.8375	9.3984	0.3100	0.5777	0.6499
Archaeospora	0.4090	15.7980	0.5193	0.4712	0.6431
Claroideoglomus	0.7278	16.7840	1.5738	0.2097	0.4721
Diversispora	-1.3748	14.0178	1.5728	0.2098	0.4721
Geosiphon	0.0000	5.5822	0.0000	1.0000	1.0000
Glomus	-0.6857	18.7090	2.6929	0.1008	0.4536
Paraglomus	0.7613	18.6467	6.5095	0.0107	0.0966
Scutellospora	3.2193	10.5885	0.8959	0.3439	0.6190
	N Fertilizer				
Acaulospora	1.2060	5.7364	0.4907	0.4836	0.9652
Ambispora	0.3215	9.1175	0.0633	0.8013	0.9652
Archaeospora	0.1353	15.6430	0.0819	0.7747	0.9652
Claroideoglomus	-0.0237	16.7823	0.0019	0.9652	0.9652
Diversispora	-0.5084	13.6338	0.2227	0.6370	0.9652
Geosiphon	-3.6210	6.8385	1.3155	0.2514	0.9652
Glomus	-0.5601	18.4693	2.2882	0.1304	0.9652
Paraglomus	-0.0612	18.8838	0.0533	0.8173	0.9652
Scutellospora	-0.6344	11.0411	0.0105	0.9185	0.9652

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

Table 4.10. Differential abundance in genus rank impacted by compartment (bulk soil vs. roots) in 2019 and 2020.[18]

Genus	logFC	logCPM	LR	P-value	FDR
	Compartment (bulk soil vs. roots)				
	2019				
Acaulospora	0.0000	5.8542	0.0000	1.0000	1.0000
Ambispora	-4.5508	13.2972	45.3874	0.0000	<0.001***
Archaeospora	-2.2978	15.5694	46.1072	0.0000	<0.001***
Claroideoglomus	1.1599	16.2402	13.6099	0.0002	<0.001***
Diversispora	0.7006	13.4025	0.6320	0.4266	0.5485
Geosiphon	-5.4037	7.2650	1.1047	0.2932	0.5278
Glomus	1.5399	17.7457	35.8181	0.0000	<0.001***
Paraglomus	0.1307	19.5759	0.2346	0.6282	0.7067
Scutellospora	-1.5090	12.1991	0.8303	0.3622	0.5433
	2020				
Acaulospora	-1.7607	6.7716	0.6778	0.4104	0.4617
Ambispora	-3.4070	11.7094	33.1994	0.0000	<0.001***
Archaeospora	-0.9622	16.5093	12.2553	0.0005	<0.01**
Claroideoglomus	0.3499	16.6182	1.5912	0.2072	0.2663
Diversispora	-0.7920	14.0620	1.6896	0.1936	0.2663
Geosiphon	-3.2432	8.4337	3.3329	0.0679	0.1222
Glomus	0.3701	18.4968	3.6675	0.0555	0.1222
Paraglomus	1.0221	18.7609	38.7068	0.0000	<0.001***
Scutellospora	-1.1254	11.9039	0.3896	0.5325	0.5325

Note: LogFC designates log fold change; LogCPM, log counts per million; LR, likelihood ratio; FDR, false discovery rate.

*, **, and *** show statistical significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

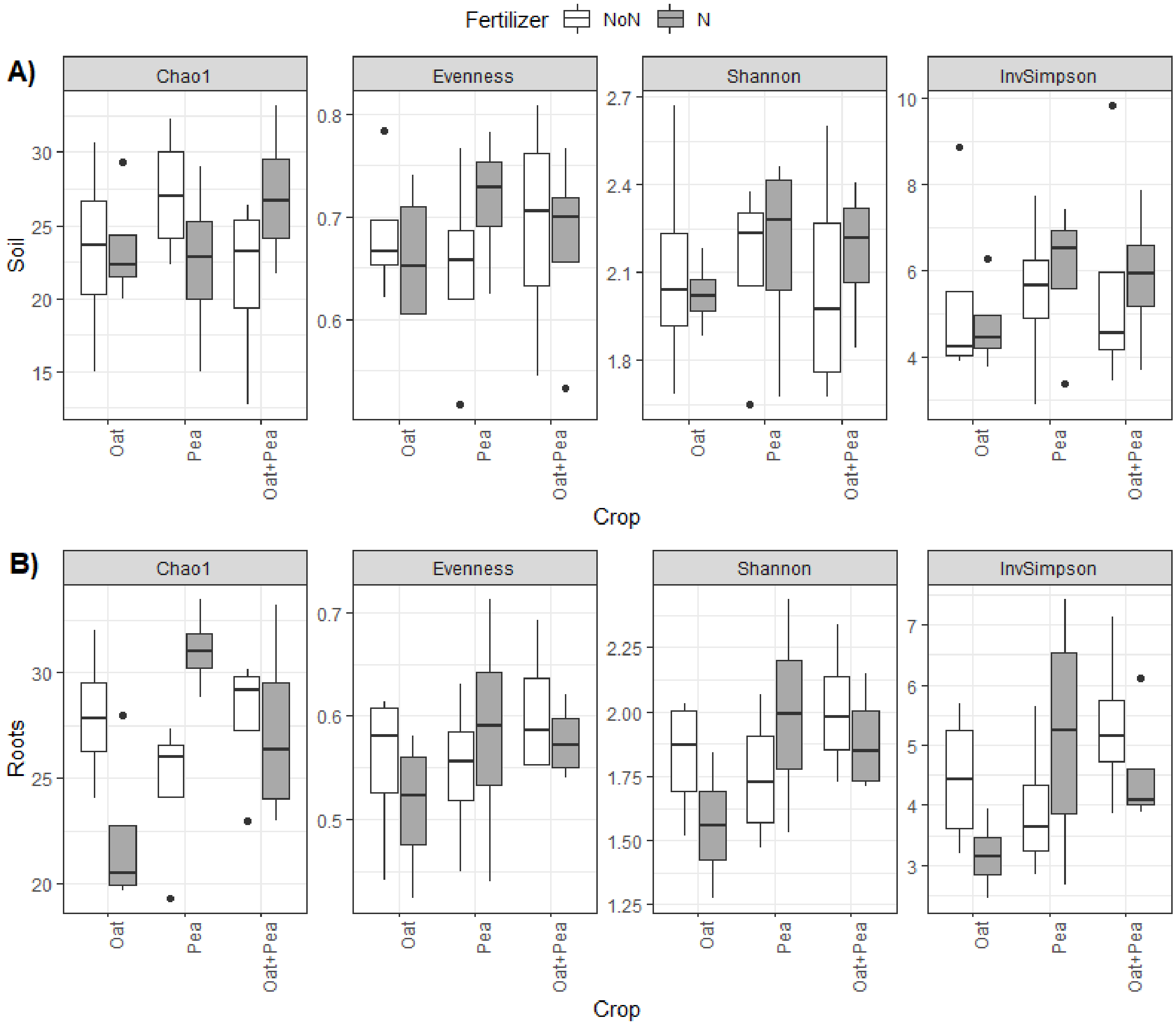


Fig. 4.1. Arbuscular mycorrhizal fungi (AMF) alpha diversity including Chao1 richness, Peilou's Evenness, Shannon diversity and inverse Simpson diversity across cropping systems at (A) bulk soil and (B) roots in 2019.[8]

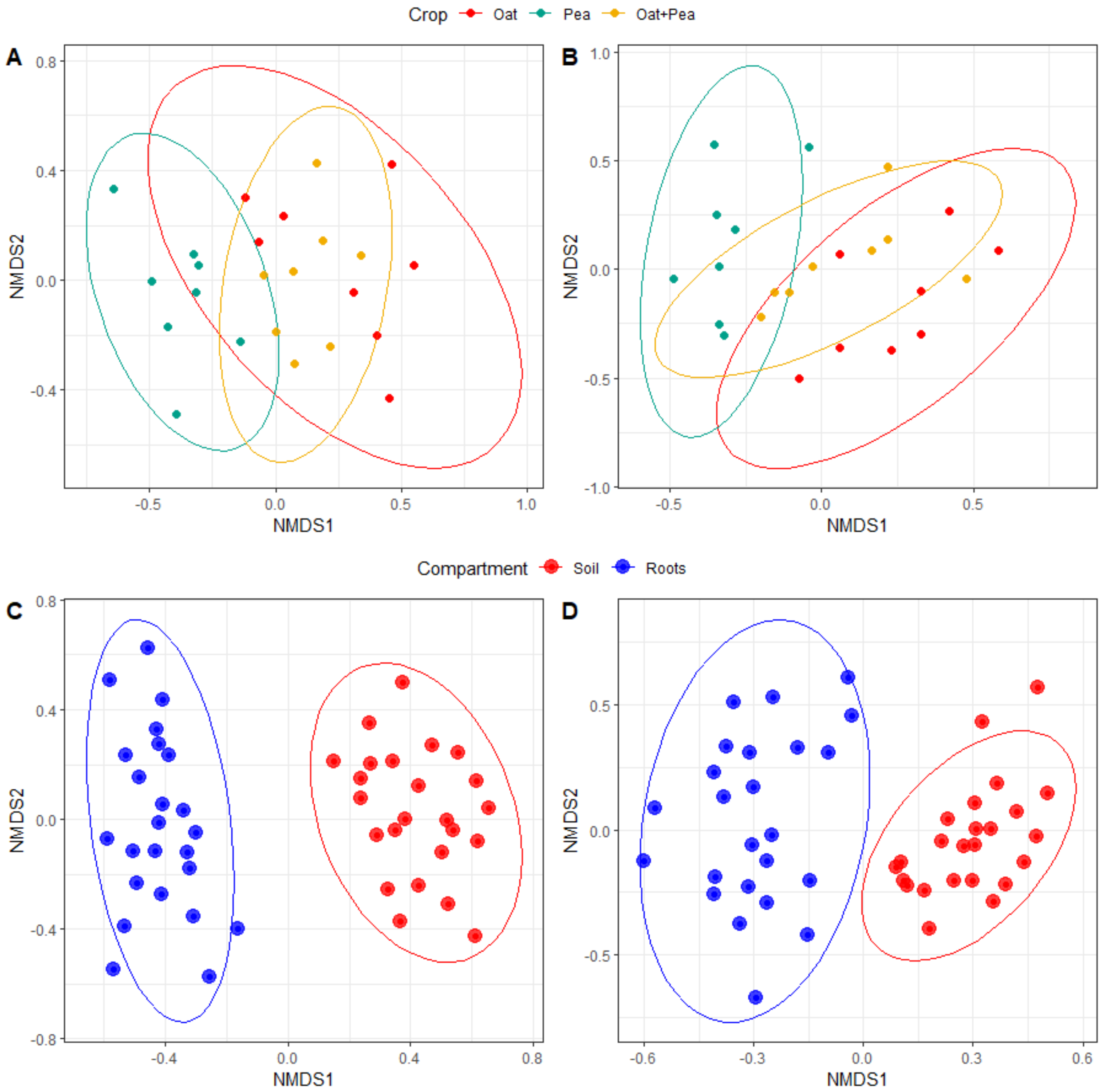


Fig. 4.2. Non-metric multidimensional scaling (NMDS) plots of AMF communities based on Bray-Curtis distances. Circles are 95 % confidence ellipses of the comparison, roots community composition significantly differs between: cropping systems in (A) 2019 and (B) 2020, compartments (bulk soil vs. roots) in (C) 2019 and (D) 2020.[9]

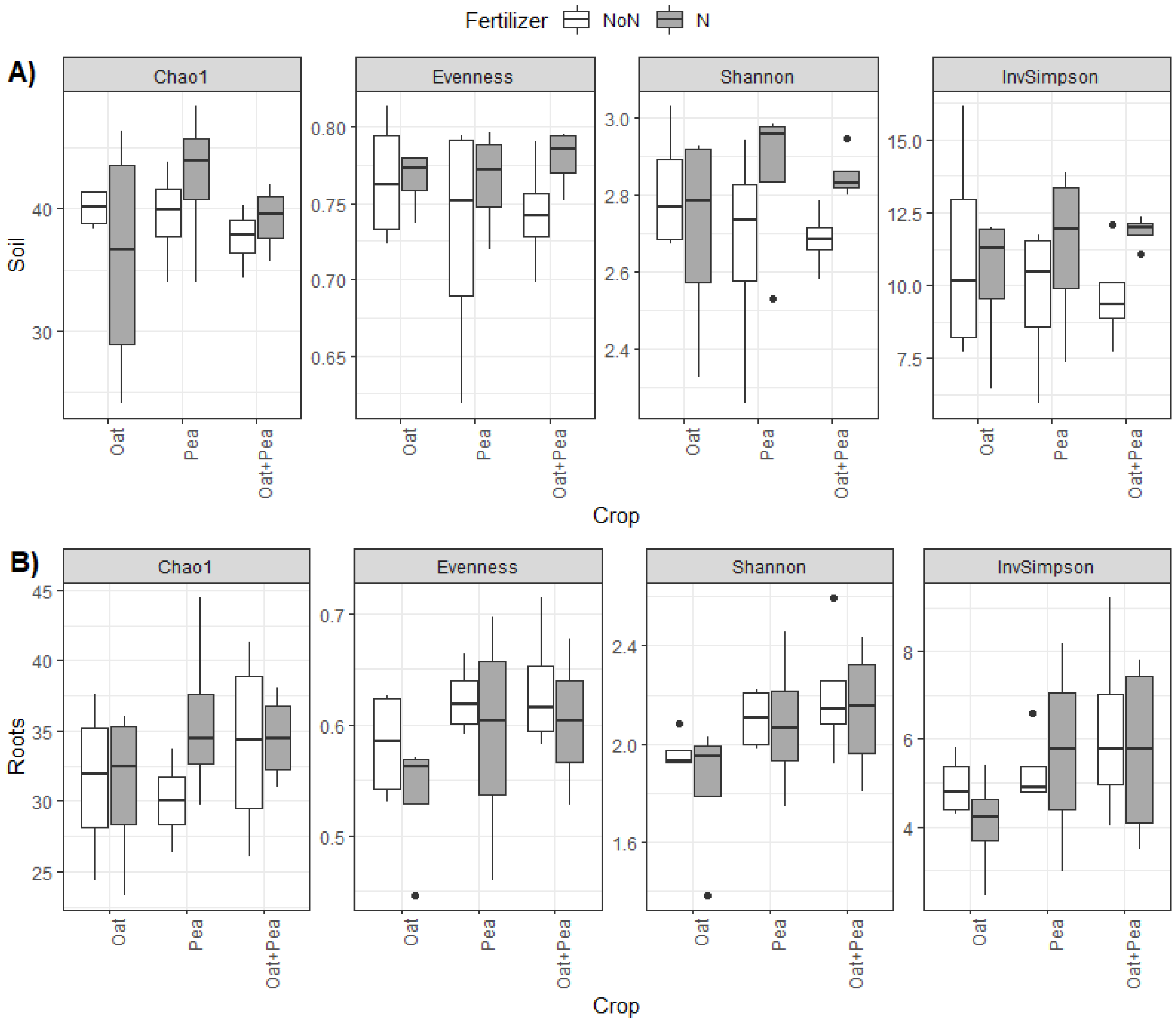


Fig. 4.3. Arbuscular mycorrhizal fungi (AMF) alpha diversity including Chao1 richness, Peilou's Evenness, Shannon diversity and inverse Simpson diversity across cropping systems at (A) bulk soil and (B) roots in 2020.[10]

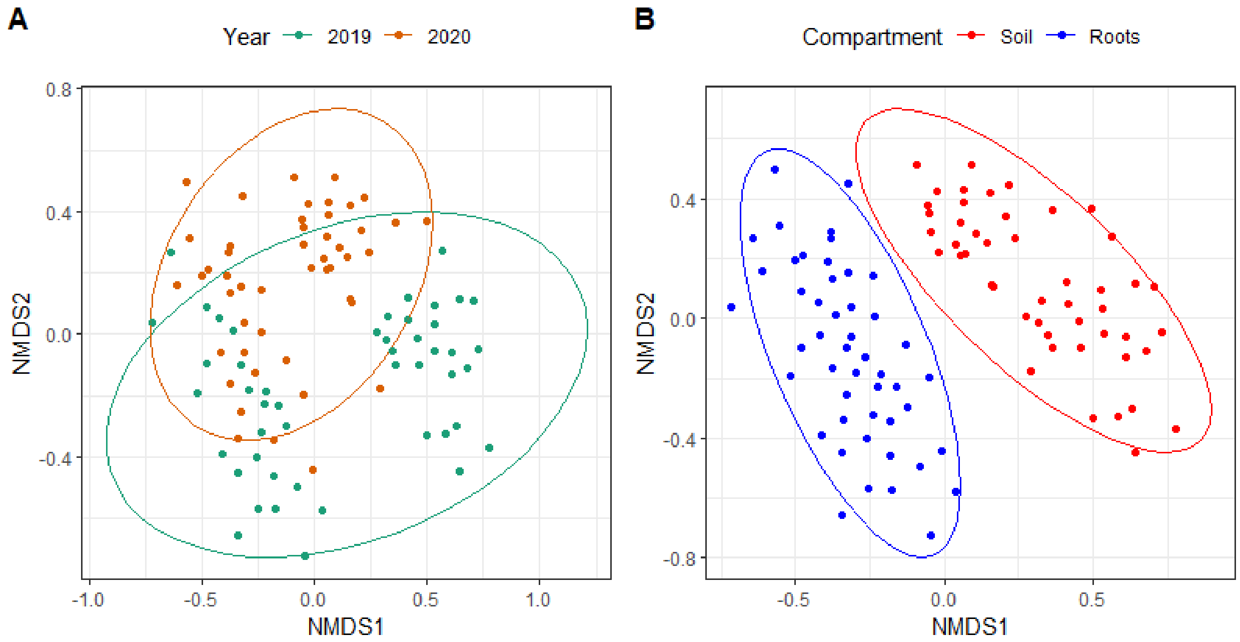


Fig. 4.4. Non-metric multidimensional scaling (NMDS) plots of AMF communities based on Bray-Curtis distances. Circles are 95 % confidence ellipses of the comparison, community composition significantly differs between; (A) years and (B) compartment (bulk soil vs. roots).[11]

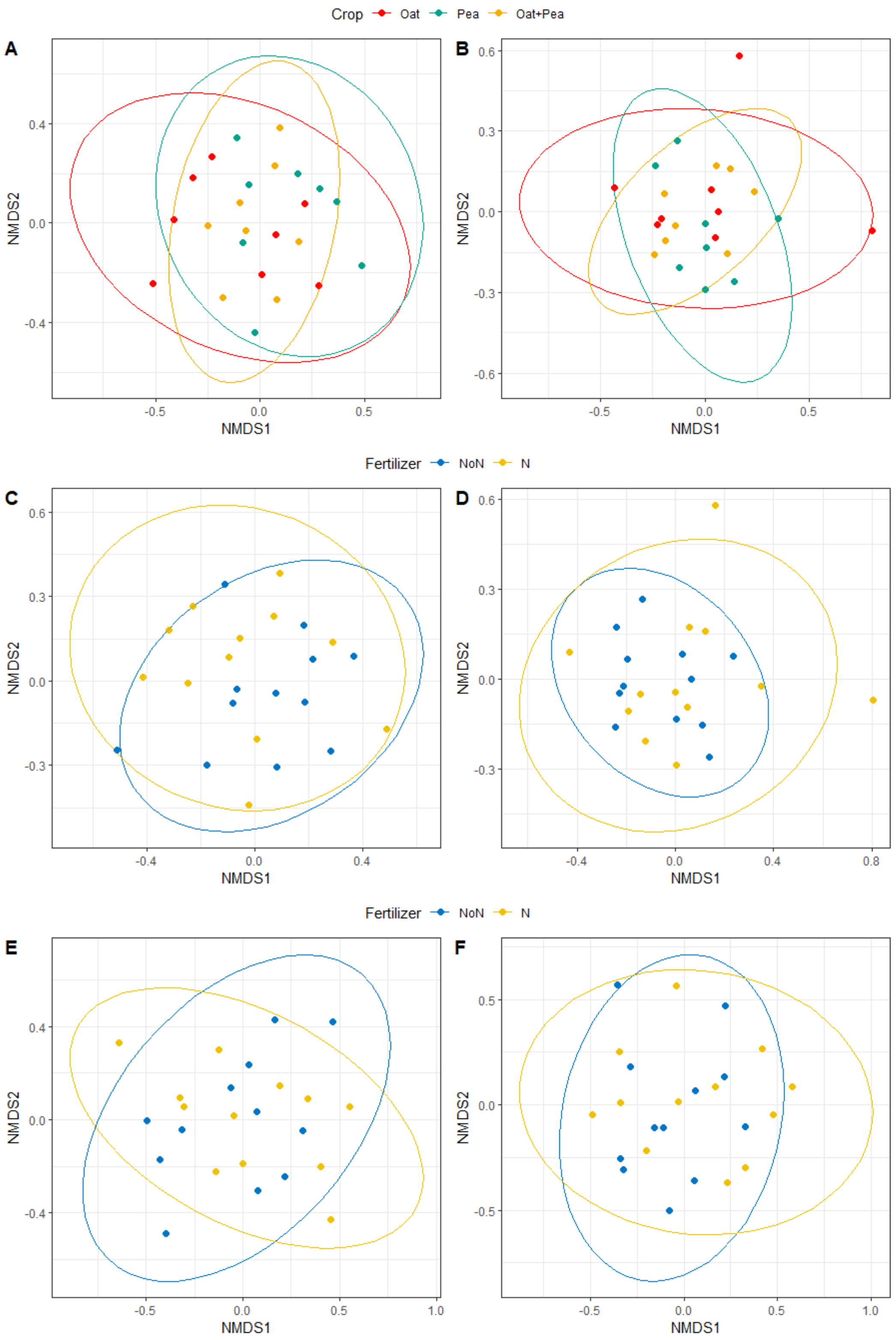


Fig. 4.5. Non-metric multidimensional scaling (NMDS) plots of AMF communities based on Bray-Curtis distances. Circles are 95 % confidence ellipses of the comparison, soil community composition significantly differs between; cropping systems (A and B) and N fertilizer treatment (C, D, E and F). Left panels indicate 2019 data while right panels show 2020 data.[12]

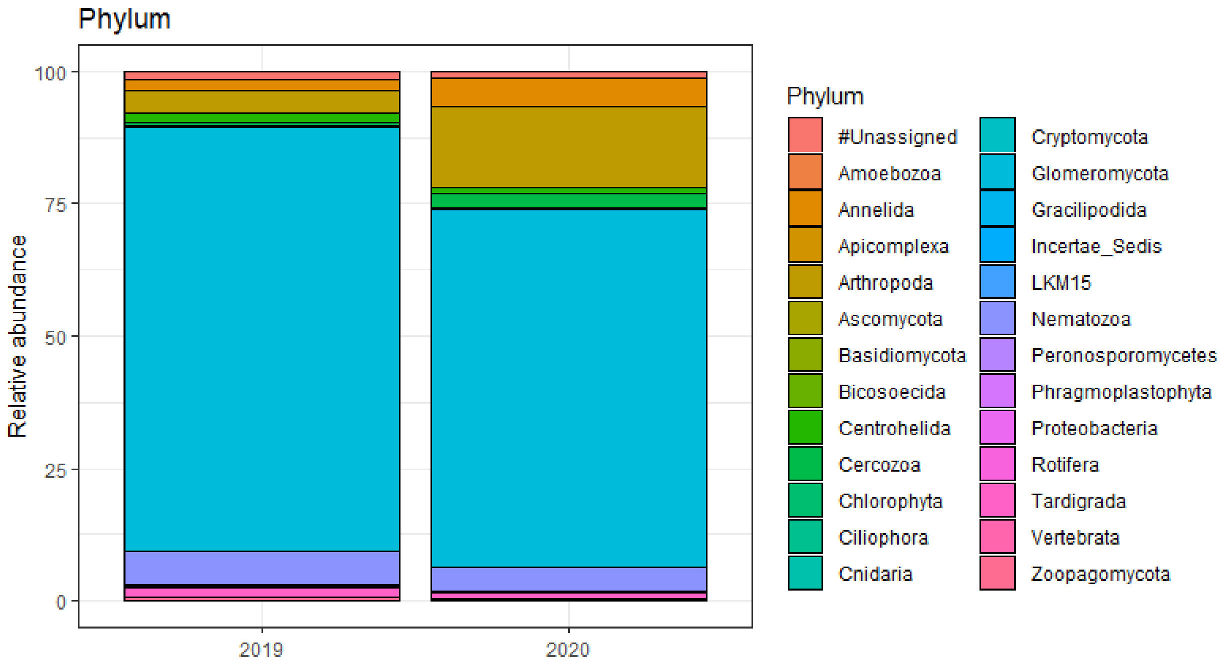


Fig. 4.6. Proportional composition of AMF communities. The panel indicates proportional sequence abundance in each of the two study years. Legend shows phylogenetic level to identified AMF phylum.[13]

2019: 80.14 % assigned to Glomeromycota, 1.50% not assigned to any Phylum
2020: 67.74 % assigned to Glomeromycota 1.23% not assigned to any Phylum

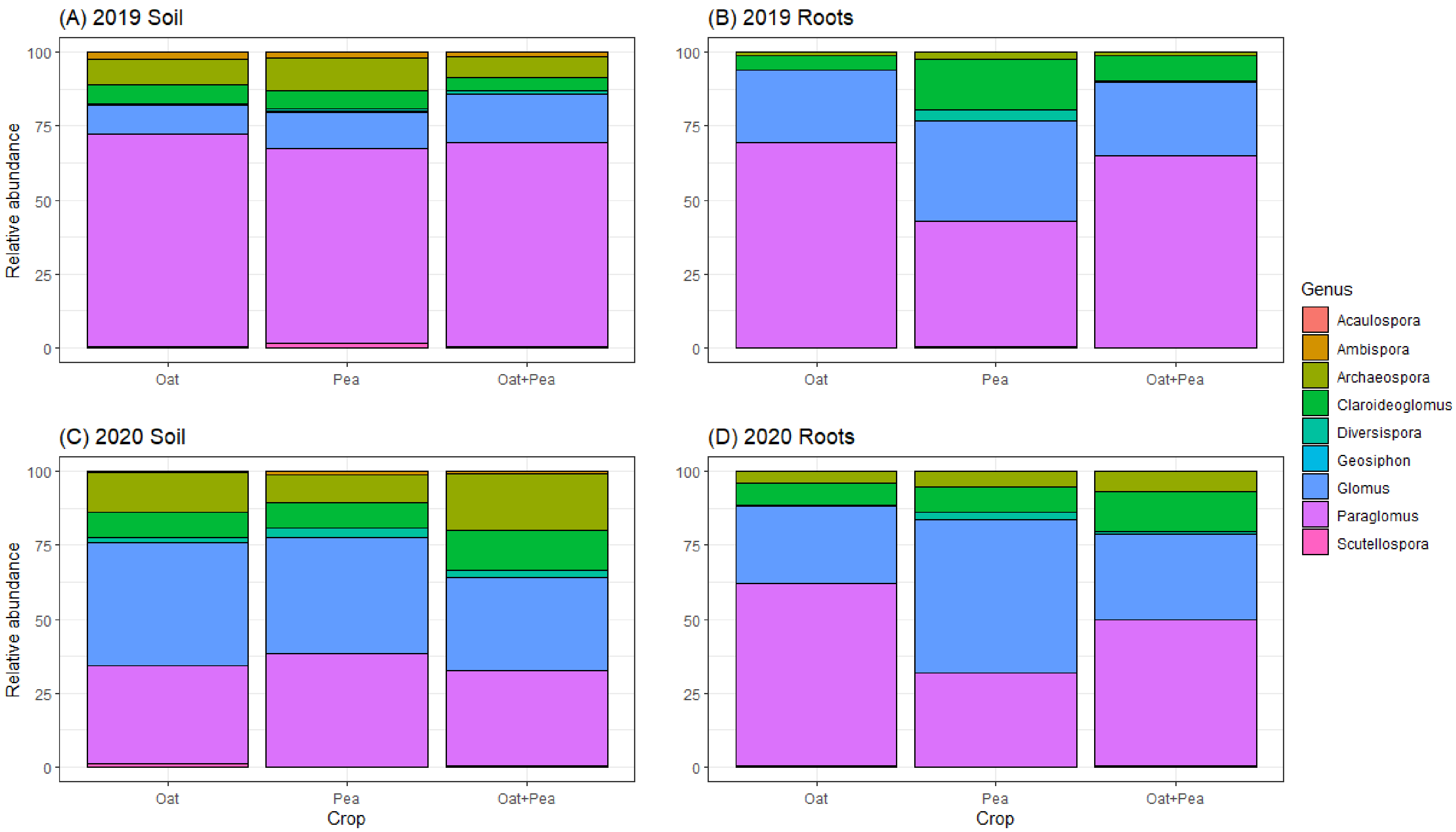


Fig. 4.7. Proportional composition of AMF root and soil communities. Panel indicates proportional sequence abundance at site. Legend shows phylogenetic level to identified AMF genus.[14]

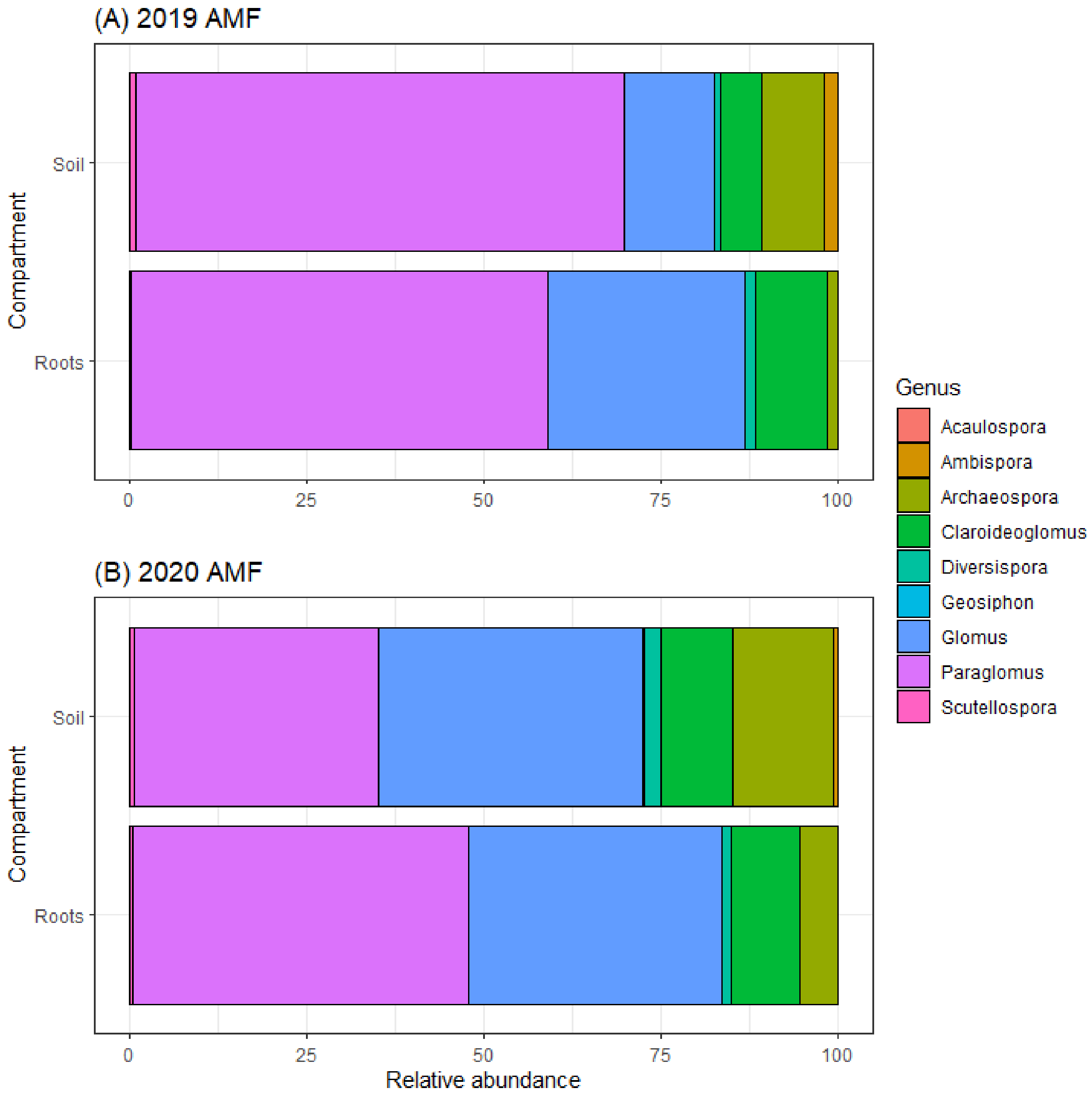
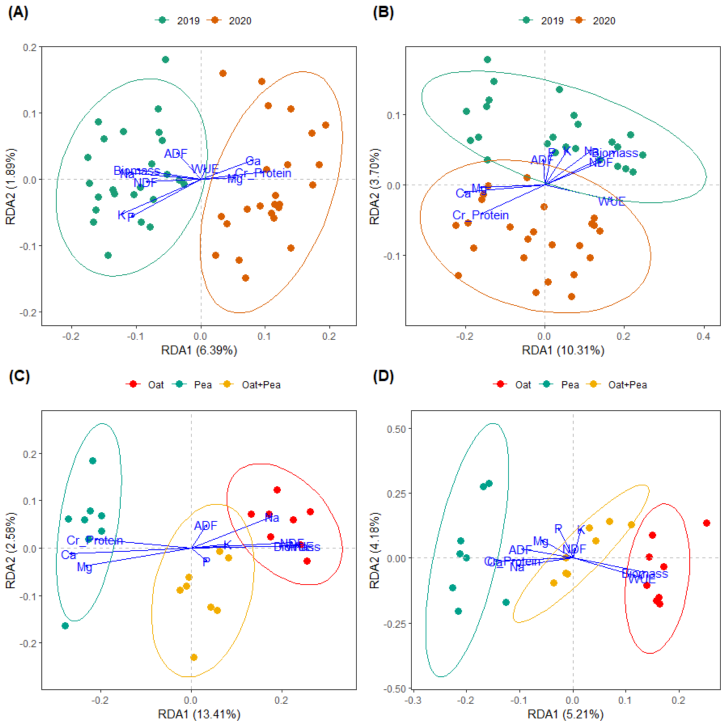


Fig. 4.8. Proportional composition of AMF root and bulk soil communities in 2019 (A) and 2020 (B). Legend shows phylogenetic level to identified AMF genus.[15]

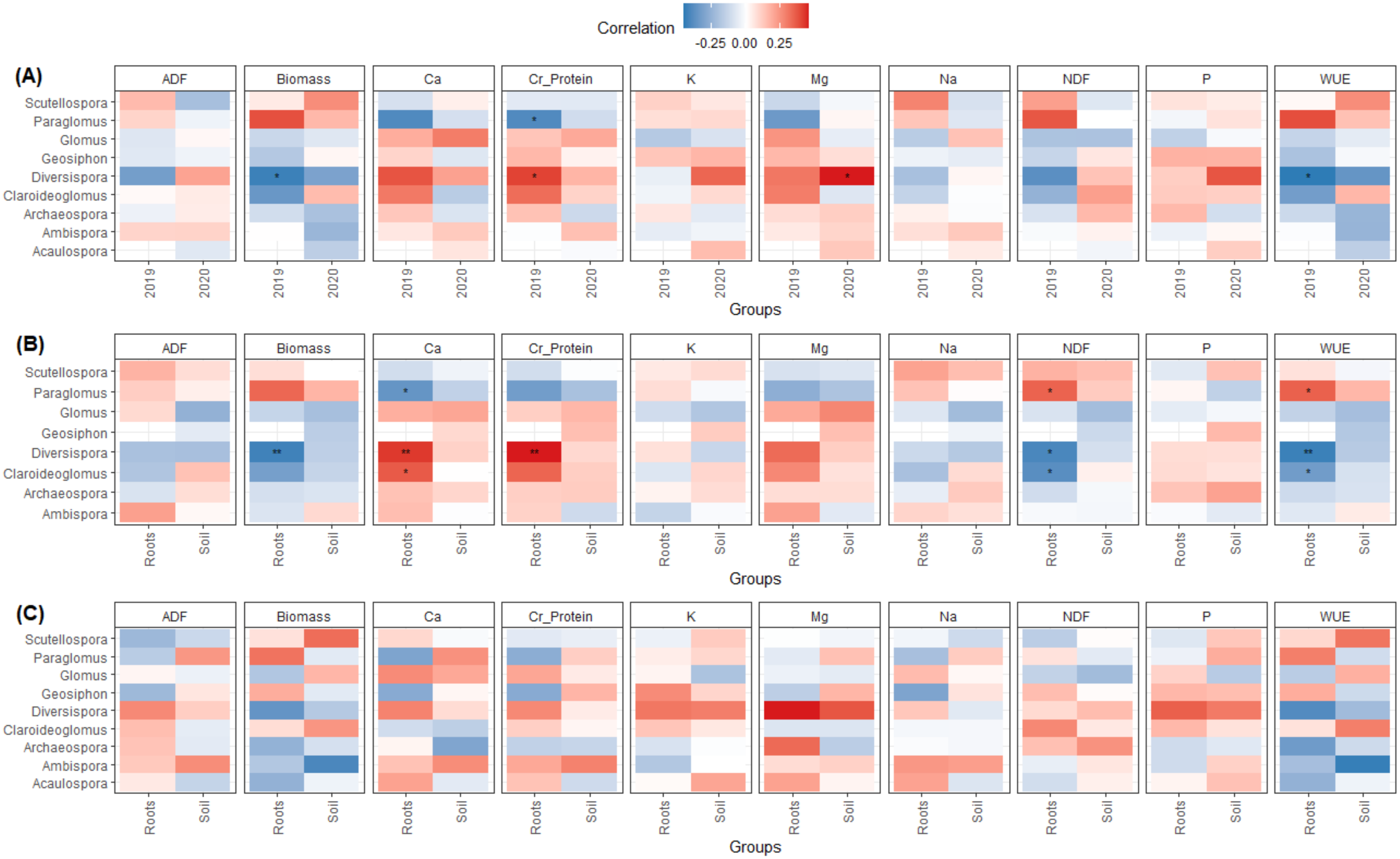
4.9 Supplementary figures and Tables



Supplementary Fig. 4.1. RDA of AMF taxonomic community composition among soil (A) and root (B) sample for two different years. RDA of AMF taxonomic community compositions across cropping treatments [Oat, Pea, Oat-Pea] in 2019 (C) and 2020 (D).[16]

Arrows in RDA graph represent significant fitted vectors.

Fitted vector	A	B	C	D
ADF	1	0.865	1	0.525
Biomass	0.102	<0.01**	<0.01**	0.63
Ca	0.130	<0.01**	<0.01**	0.496
Cr_Protein	0.084	<0.01**	<0.01**	1
K	<0.01**	<0.05*	1	0.16
Mg	1	<0.01**	0.01	1
Na	<0.05*	<0.05*	<0.05*	1
NDF	0.168	<0.01**	<0.01**	1
P	<0.01**	0.212	1	0.198
WUE	1	<0.05*	<0.01**	0.695
Model	<0.001***	<0.001***	<0.001***	<0.01**



Supplementary Fig. 4.2. The heatmap of the correlation between AMF genus rank and physiochemical characteristics of (A) two years (2019 and 2020) and (B) compartments in 2019 and (C) compartments in 2020. Spearman's correlation analysis was used for creating the heatmap. Positive correlation is shown in red, whilst negative correlation is shown in blue. The asterisks mean significant correlations (*, $p<0.05$; **, $p<0.01$).[17]

Chapter 5: General Discussion and Conclusion

The increase in global population is a major challenge for the agriculture as more sectors would compete for available water resources (FAO 2011), and agriculture would nonetheless need to increase production to match global food demands (FAO/OECD 2018). Currently, one of the solutions is to sustainably increase fertilizer usage to avoid fertilizer losses (Hendricks et al., 2019; Liang et al., 2021; Qiang et al., 2020), as well as optimizing N fertilization (Thilakarathna et al., 2020, Chai et al. 2020). Selection for better genetics could lead to better yields, but this is limited by incomplete knowledge of what "better genetics" is (Guo et al. 2021). However, as these methods reach its upper limits, each optimization would have diminished yield gains.

Intercropping had been re-examined as an alternate method to increase yield. Through spatial arrangement, crop species selection, and harvest timing (Du et al. 2016, Zhang et al. 2008, Yang et al. 2018), intercropping can more efficiently use the resources present in the field compared to monocropping, thus potentially reducing the necessity for increased fertilizer use and genetic modification.

Recent studies have found that intercropping has different ways to improve overall yield. The overyielding effect, where the plants can yield more than its monocrop counterpart, is the common findings in these studies (Xiao et al. 2019). The potential reason for overyielding is attributed to potential plant-plant and plant-soil interactions. For example, Brooker et al. (2016) found that legumes in intercropping promotes nitrogen (N) uptake by intercropped plant species, and this increase in N uptake subsequently leads to enhanced nutrient balance, and ultimately biomass production (Egan et al. 2019; Diaz et al. 2006). Additionally, intercropping improves water use-efficiency (WUE) (Caviglia et al. 2004; Gao et al. 2009; Ren et al. 2016). Within some recent studies, there is evidence pointing towards mycorrhizae as a facilitator of better water and nutrient use efficiency (Bever et al. 2009; Hauggaard-Nielsen and Jensen 2005; Koide 1991; Li et al. 2014). That said, these recent studies demonstrate that intercropped plants are more resilient than when they are cropped alone.

This two-year project demonstrated the effects of intercropping on forage crops, specifically oat, pea, and canola. We found that intercropping does not always yield more than monocrop systems but yields more consistently. There was also more stability in terms of forage nutritional indicators and mineral contents (Chapter 2). We also found that intercropping can improve WUE, but noticed that higher water uptake does not consistently translate to improved WUE (Chapter 3). Finally, we identified key mycorrhizal genera present in the rhizosphere, indicating that they may be factors in improving crop yields (Chapter 4). In this conclusion chapter, I will examine each finding and explore how they can be studied further.

5.1 Forage Yield and Nutritive Indicators

Intercropping can yield more than monocropping in terms of forage yield, but as mentioned in Chapter 2, there are specific environmental conditions that help intercropped systems perform better (Pflueger et al. 2020, Han et al. 2012), and this project demonstrated that nitrogen fertilizer addition and seasonal precipitation can affect the performance. Moreover, when observing the nitrogen utilization efficiency (NU_tE), we found that intercropping produces more yield per unit of nitrogen present in general. Therefore, by studying what the optimal conditions are for intercropping systems, we can utilize its high NU_tE potential to increase forage yield.

As tools to measure normalized difference vegetation index (NDVI) and photosynthetic efficiency of photosystem II (Y[II]) become more available, it becomes a very effective proxy tool to estimate crop performance. In Chapter 2, our regression found that 50% of the variations in NDVI could be explained through plant yield and nutritional indicators (Htoon et al. 2014).

One speculation for what could also alter NDVI values was the nutritional acquisition strategy the plants would be using under different environmental conditions (Simunek and Hopmans 2009), as we observed a smaller difference in margins in NDVI in the wetter 2020 study year. We also found that nitrogen fertilizer addition does not significantly influence NDVI values, and that the oat-pea and peacanola intercropping systems not only had high NDVI but forage biomass yield as well, which makes these systems promising alternatives to their monocropped systems. We found no noticeable difference in photosynthetic efficiency Y[II] across treatment combinations. We believe that as the fluorometer only captures a fixed portion of the leaf when measuring Y[II], there needs to be a metric such as leaf area index (LAI) to fully realize the plant's photosynthetic efficiency.

In terms of forage quality, the important step for forage is to meet the required level of feed nutrition for the livestock. In this project, the targeted livestock was beef cattle. There were no significant effects from adding nitrogen fertilization. Intercropping systems in this project, along with monocropping systems, met most of the mineral and energy requirements (crude protein, total digestible nutrients, acid detergent fibre, neutral detergent fibre, net energy for gain, etc.) for beef cattle (NASEM 2016). There were certain cases where the intercropping system significantly improved digestibility. For example, we found that pea-canola intercropping had significantly lower neutral detergent fibre (NDF) content than pea alone or canola alone. With lower NDF values, this would mean the forage is more digestible, which in turn would mean a more attractive feed to cattle. There is limited knowledge explaining why intercropping can improve digestibility. An in depth understanding of this nutritional aspect, paired with the known fact that digestibility increases by harvesting forage during its earlier stages (Hoffman et al.

2001), would become key to fully unlock the potential of forage production in intercropping systems.

5.2 Water use efficiency

By determining water use efficiency (WUE), we were able to examine certain plant behaviors in an intercropped system (Chapter 3). Biomass WUE (WUE_{BM}) increased with greater moisture available in pea-canola intercropping, while WUE_{BM} increased with lesser moisture available in oat-pea intercropping. In terms of crude protein yield, protein WUE (WUE_{CP}) was measured. Oat-pea intercropping produced poor WUE_{CP} likely because of generating high biomass yield; a phenomenon termed a dilution effect (Jarrell and Beverly, 1981). Comparatively, this effect was not present in the pea-canola intercropping system, where we believe that due to their taproot structures, there was less competition as root development in taproots is predominantly vertical than horizontal.

Despite having high WUE, an intercropping system did not necessarily improve water uptake characteristics, as they often use less water for similar yields (Chapter 3). Furthermore, as abovementioned, plant root structure and synergy within intercropping systems play a major role in determining how water uptake occurs. We noticed that water uptake is highly dependent on the cropping system in drier growing seasons, as we found no significant interactions between nitrogen fertilizer addition and intercropping systems, as well as no significant interactions between rates of nitrogen fertilizer addition. Conversely, in a wetter growing season, we found that nitrogen fertilizer addition significantly alters water uptake, such that nitrogen fertilizer addition increased water uptake. We also discovered oat, pea, and canola water uptake response to nitrogen fertilizer under wetter growing seasons. Oat consistently increased water uptake with fertilizer addition throughout the growing season, while pea consistently decreased water uptake, but canola removed less water in the first half of the growing season and more water in the second half. We believe that the fast development of oats contributes to its capability to increase water uptake in the presence of nitrogen fertilizer, and because the pea-rhizobia symbiosis lessens in the presence of nitrogen fertilizer, less water was also removed to facilitate nitrogen fixation. In the case of canola, the application of 75 kg urea-N ha^{-1} may have been impeding early growth due to its high salt content. While we hypothesize that the main driver to water uptake behaviors and WUE is root structure and synergy between crop species, there is a need to further confirm that plants only uptake from the measured soil surface and the subsurface layers. It is possible that plants may have accessed deeper water reservoirs from soil layers beneath our soil sensor deployment. As well, since the soil surface could dry out faster than plant water uptake due to evaporation early in the season, the shallow soil sensor measurement might be inaccurate.

5.3 Mycorrhizal synergy

We examined mycorrhizal symbiosis in oat-pea intercropping (Chapter 4), where we found significantly different arbuscular mycorrhizal fungi (AMF) communities between sole oat, sole pea, and oat-pea intercropping. One major key finding is that by intercropping oat and pea together, mycorrhizal alpha diversity index is higher than sole pea or sole oat. We believe this is caused by the two plant species recruiting mycorrhizal species suited for their individual needs, thus leading to an overall increase in diversity despite potential overlap in species (Broekling et al. 2008). Additionally, nitrogen fertilizer application also determined species abundance in the system. Oat alone and intercropped with pea saw a decrease in mycorrhizal abundance, but pea saw an increase in mycorrhizal abundance. We believe that the readily available source of nitrogen provided by fertilization alters the nutrient acquisition strategy that the plants naturally have, thus causing the shift in abundance. While this topic is often discussed in broad detail in other studies (Hodge and Storer 2015; Johnson et al. 2015), individual plant species nutrient uptake strategies under different rates of nitrogen fertilizer are a less explored topic and would help expand on how plant mycorrhizal recruitment occurs under intercropping systems.

Besides examining how mycorrhizal diversity and abundance is altered in intercropping systems, we also identified key mycorrhizal genera present in the individual crops. *Diversispora* was consistently more abundant in oat alone and oat-pea intercropping compared to pea alone. *Clarideoglomus* was found to be more abundant in pea alone in the drier year of the project (2019), which led us to believe that this genus is likely recruited for moisture acquisition purposes. Paraglomus was observed to decrease in relative abundance in a wet year in pea alone and oat-pea intercropping. We hypothesize that this genus is discouraged by root exudates from pea roots to be recruited as high moisture could be a stress factor for pea growth (Karst et al. 2017, Nian et al. 2002), and this mycorrhizal genus might impede its root function in this type of condition, either by competing for the moisture resource or restrict the plant from accessing nutrients needed for water uptake. However, there is limited studies on the genera mentioned above, and would require extensive research to understand what their role is in the system and how the role is performed.

5.4 Future directions and conclusions

This project has increased the insights to how intercropping benefits a producer in terms of forage yield and quality, water use, and the role of mycorrhizae in facilitating the benefits. This project demonstrates the benefits of intercropping, and starts shedding light into certain underlying explanations as to why the benefits occur. By identifying key mycorrhizal genera, this project has provided a direction for how to determine what they do for the crops, and what conditions they need to thrive, thus improving how intercropping can be implemented to match the criteria for a better soil condition that can lead to the benefits observed in this project. Furthermore, by identifying that root structure also plays a role in increased efficiency for water uptake, we can explore how to spatially locate each plant to best maximize the relationship between two plant species, thus also increase land resource use efficiency. I believe that by expanding on these findings, we would be able to provide producers a reliable best management practice that would reduce input cost while at the same time would improve soil conditions.

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