Soil Health and Crop Connectivity in Barley-Pulse Intercropping Systems

A Thesis
Presented in Partial Fulfillment of the Requirements for the
Degree of Master of Science
with a
Major in Environmental Science
in the
College of Graduate Studies
University of Idaho
by
Torrey J. Stephenson

Approved by:
Major Professor: Zachary Kayler, Ph.D.
Committee Members: Xi Liang, Ph.D., Jan Eitel, Ph.D.
Department Administrator: Leda Kobziar, Ph.D.

August 2022

Abstract

Soil health is a necessity for a sustainable future from food security to ecosystem health. Nearly 38% of the earth's land surface is farmed, meaning agriculture has an enormous role in maintaining the capacity of soil to sustain plants, animals, and humans. We are faced with the challenge of increasing production on this land to support current and projected population growth without jeopardizing the future by degrading our soil. This challenge is exacerbated by climate change and instability leading to changes in weather patterns, temperature, and precipitation regimes worldwide. In Idaho, producers face challenges such as drier summers, higher temperatures, and extreme precipitation, leading to increased demand for limited water resources. As such, there is a clear need to investigate growing strategies that are resilient to water stress and advantageous to soil health.

The production of barley and nitrogen-fixing pulse crops such as lentils and peas, often in rotation, is already a staple of Idaho agriculture. This research investigates the effects of growing barley and pulse crops together simultaneously (intercropping) on soil health, focusing specifically on nutrient status and soil microbial communities. To understand the role water limitation will play in intercropping, soil health was measured under full and deficit irrigation conditions.

We demonstrated that complementary root characteristics in barley/pulse intercropping allow more efficient use of water and nitrogen in the soil profile, as barley accesses deeper resources than pulse crops. Pulse crop biomass was significantly reduced under both irrigation strategies in intercropping; barley yields were less impacted, likely because barley outcompeted pulse crops for soil resources. Barley's ability to outcompete pulses is enhanced under water stress: in the deficit irrigation treatment, the decrease in intercropped barley biomass was not significant in comparison to monocropping, though the pulse biomass decrease was. Intercropping additionally altered the soil microbial community, overall increasing diversity as compared to monocropping barley. This shift was especially apparent for barley/lentil intercropping.

The response of barley to intercropping with peas as compared to lentils was not identical. Intercropping barley with pea resulted in greater relative barley biomass than intercropping with lentil, likely due to increased nutrient availability. Stable isotope tracing of pulse crop nutrient allocation suggests that peas release a greater proportion of fixed carbon and nitrogen to the soil than lentils, increasing nutrient access to companion crops and microbes. Further, we demonstrated the

short-term connectivity of barley and pulse crops grown together, with nitrogen fixed by pulse crops detected in the roots of companion barley crops within the range of several days.

Significant changes in soil health metrics observed after a single growing season are a positive indication that intercropping barley and pulse crops is a strategy that could benefit producers. Data obtained from subsequent growing seasons will further clarify the longer-term effects of intercropping and water availability on soil nutrients, microbial community, and productivity. This work can help to inform management decisions such as inputs and crop selection for producers aiming to employ diverse cropping strategies and improve soil health.

Acknowledgments

I would like to acknowledge my advisor, Dr. Zachary Kayler. I am grateful to have found in your lab an environment that has deeply challenged me and allowed for unimaginable growth. In the last two years, I have learned to take calculated risks, pursue my curiosity, solve problems creatively, persevere, and trust both myself and the process. Thank you for your patience even when my writing has no Roter Faden, the long and often whirlwind conversations, supporting my strange side projects, and the innumerable opportunities to grow.

Dr. Xi Liang always had a soil moisture sensor ready and waiting to welcome me to Aberdeen! Thank you for sharing so much of your expertise and doing the hard work to plant, manage, and harvest the plots. I am grateful to have had the opportunity to learn a bit about the agronomic side of our research, which deeply enhanced my understanding. I am also appreciative of Dr. Jan Eitel. When I was buried in the minute details of my research, your thoughtful perspective reminded me to zoom out and reflect on the nature of research and knowledge at a larger scale, and to remember the excitement of discovery.

Isa von Rein took me under her wing when I arrived and was always willing to answer questions and share what she knew, asking for nothing but cute cat photos in return. Thank you for welcoming me to the lab and walking me through so many procedures with endless patience.

Thanks also to the students whose efforts were instrumental in this research: Peter Lin, Nolan Frampton, Paige Martin, and Chynah Zamorah.

Dedication

I am endlessly grateful to a number of people who were instrumental in my path to the University of Idaho and making it home once I arrived.

First, to my soil-mate Emily—your mentorship and support started this journey and has forever changed my trajectory.

To everyone who got me through the hard days and were the reason behind the best days—the Thursday night cribbage matches, the mountain bike adventures, the frizz coffees, the board game nights, and so much good food. Thank you. In particular, to the best lab mate and partner in mischief I could have asked for—may you always be on EHS's good side.

To my favorite bridge troll, who is incredible enough to sweep me off my feet despite stiff competition from the EA-IRMS.

And, of course, to LP—I literally could not have done it without you.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Importance of soil health

The soil beneath our feet is the source of the vast majority of our food as well as the foundation of untold ecosystem services that regulate our water supply, mitigate climate change, and drive the global cycling of nutrients. Maintaining soil health, defined as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" is essential for the health of the atmosphere, lithosphere, hydrosphere and biosphere (Lehman et al., 2020).

Within this broad definition, soil health is often considered for its essential role in crop production and global food security. The global population is expected to increase from approximately 7.9 billion people today to 9.7 billion by 2050, and 10.9 billion by the turn of the next century (Census.gov; United Nations, 2019). Supporting this population growth will require huge increases in agricultural production to supply adequate food, fuel, and fiber.

Within the last 100 years, dramatic intensification of crop production (the "Green Revolution") has already occurred. Largely due to technological advances including the advent of high-yield crop varieties and synthetic inputs such as fertilizers and pesticides, farm output from 1948 to 2015 increased by 170% (Wang, Nehring, & Mosheim, 2018). This productivity has come at a cost to soil; 33% of the soil covering the earth's surface has been degraded chemically, biologically, physically, or ecologically (Lal, 2015). This degradation threatens the sustainability of future crop production, as well as human and ecosystem health more broadly (Yang, Siddique, & Liu, 2020).

The role of climate change

Agricultural production variables such as yield and inputs are tightly linked to the climate, particularly as it influences water availability. Low precipitation during the growing season means that much of the agriculture in Idaho, particularly in the Snake River Plain, relies on stored water for crop irrigation.

Human-induced climate change has adversely impacted the health of ecosystems and humans worldwide, though the specific impacts vary by region (IPCC, 2022). Climate projections for Idaho include a higher likelihood of extreme/infrequent precipitation events, drier summers, and higher temperatures, all of which culminate in decreases in plant-available water and increases in irrigation demand ("Economic Impacts of Climate Change on Agriculture in Idaho," 2018). The emission of greenhouse gases such as carbon dioxide (CO_2) and nitrous oxide (N_2O) also play a role in exacerbating water limitation through their influence on temperature.

Levels of atmospheric CO_2 have been rising continuously since the industrial revolution, increasing by 131.6 ppm between 1750 and 2019 (IPCC, 2021). This increase has exacerbated the "greenhouse effect," trapping heat close to the Earth's surface and increasing the global average temperature (Zhong & Haigh, 2013). In many regions, warmer temperatures will lengthen the growing season, which has the potential to increase the yields of some crops (Linderholm, 2006). However, these increases will only be realized if there is enough water to support them. Warmer temperatures also increase evapotranspiration and decrease plant-available water, which in extreme cases can negatively impact dryland farming yields (Goyal, 2004). For irrigated production, increases in water demands may exacerbate existing water shortage issues.

Curbing the rise in CO_2 emissions will help to stabilize the global climate. Plants, including crops, have an undeniable role in global atmospheric CO_2 concentrations. Annual decreases in atmospheric CO_2 are observed during the northern hemisphere's spring and summer months because of the magnitude of photosynthesis (Keeling et al., 2005). This conversion of CO_2 to organic forms in plant biomass and rhizodeposition (C sequestration) not only removes CO_2 from the atmosphere, but also increases soil organic carbon (SOC), a key to building soil health (Lal, 2014).

N_2O is an often-overlooked greenhouse gas with a warming effect nearly 300 times greater than that of CO_2 (IPCC, 2014). Approximately 50% of anthropogenic N_2O emissions are from agricultural soils, primarily due to the addition of N fertilizers (Shcherbak, Millar, & Robertson, 2014). While critical for plant growth in most regions, overapplication or mistimed application of fertilizers can have serious environmental consequences. For example, 1% of N added as fertilizer is transformed into N_2O that is released into the atmosphere, illustrating how agricultural production strategies can play a role in climate change (Stehfest & Bouwman, 2006).

Agricultural strategies that are sustainable for both people and the environment should therefore incorporate practices which promote soil C sequestration, reduce fertilizer N reliance, and are more tolerant to water-stress conditions. Guiding future production and land use decisions requires understanding and quantifying soil health, and how it is impacted by both climate change and various management strategies. This knowledge is vital for securing the future of our food supply and the health of ecosystems globally. Soil is a resource that is not recoverable on the scale of human lifetimes, but soil health is.

Pulse Crop Health Initiative

The research in this thesis was conducted as part of the Pulse Crop Health Initiative (PCHI) research project funded by the USDA Agricultural Research Service (ARS). The PCHI project combines

agronomic and biogeochemical perspectives to understand the impacts of growing pulse crops alongside barley at the same time, a practice called intercropping. The four aims of this research are to: (1) Determine soil health and plant physiology of lentils, chickpeas, and dry peas grown in rotation and intercropped with barley. (2) Determine simultaneous carbon assimilation and seasonal carbon allocation to seeds, roots, and stems. (3) Evaluate the effect of including pulses on barley production. (4) Assess the impact of water stress on pulse-barley production and soil health indicators (e.g., organic matter and microbial community). Chapter 2 of my thesis will primarily address soil health aspects of objective (1) and objective (4). Chapter 3 utilizes stable isotope techniques to elucidate plant physiology differences of objective (1) and objective (2). Analyses of grain yield and aboveground production were conducted for a separate paper; these results are used to contextualize findings but are not detailed in this thesis.

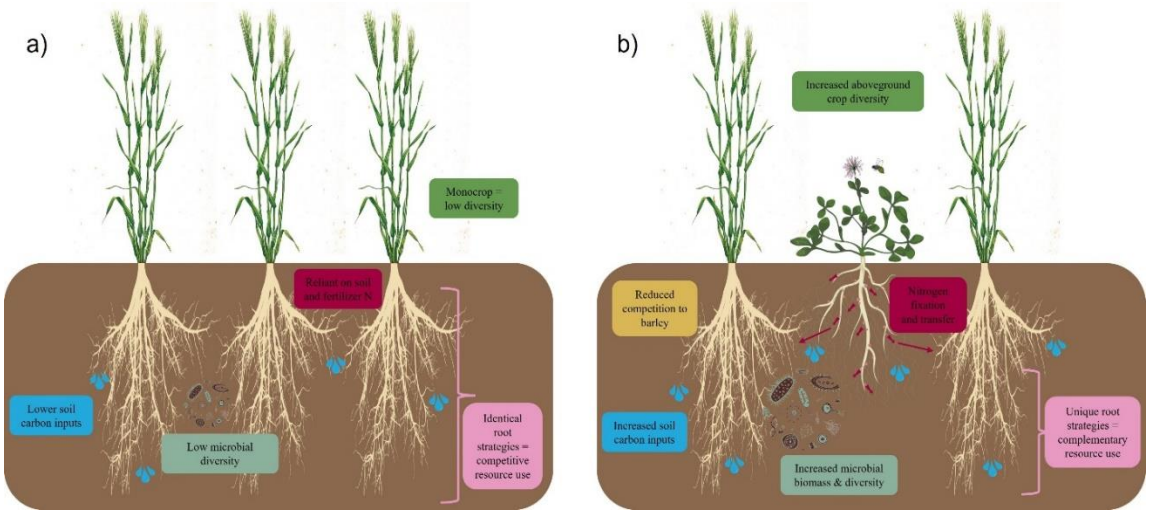

Figure 1.1: Hypothesized benefits of intercropping barley and pulse crops (b) as compared to growing sole crop barley (a). Increasing aboveground crop diversity is likely to increase belowground microbial community diversity and biomass. Nitrogen-fixing pulse crops will reduce nitrogen (N) competition with barley, and potentially increase N supply to companion crops. Greater soil carbon (C) inputs are associated with increased soil health. Shallow-rooted pulse species and deeper-rooted barley crops may extensively access resource pools (i.e. water and N) in the soil profile, further decreasing competition and promoting more efficient resource use.

Rationale

The buildup of soil organic matter and belowground microbial communities forms the basis of soil health in agroecosystems and can also lead to efficient crop use of water and nutrients. Pulse crops are good candidates to enhance soil health due to their capacity to fix both carbon through photosynthesis and nitrogen through symbiosis. Soil health is enhanced by carbon and nitrogen inputs from root turnover and labile root exudates that drive microbial community composition and soil organic matter build-up (Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013; Rasse, Rumpel, & Dignac,

2005). There is evidence that intercropping accumulates more SOC than sole cropping; this increase in C sequestration promotes both soil and atmospheric health (Cong et al., 2014).

Furthermore, a diverse microbial community improves the availability of soil water and nutrients, for example, by increasing the spatial access of rhizosphere resources through fungi and fixing N through rhizobia (Chen, Zhu, & Zhang, 2003; Strickland & Rousk, 2010). Soil health and water use efficiency are thus expected to increase when pulses are incorporated into cropping systems (Figure 1.1). Including pulses in cereal-dominant cropping systems may improve overall soil health and plant growth, but before recommendations can be made, robust estimates of soil organic matter accumulation, carbon quality, and microbial community composition are needed.

Experimental Design

This research was conducted at the University of Idaho's Aberdeen Research and Extension Center, located in the Snake River Plain of southeastern Idaho. This region of the state is known for irrigated farms producing crops such as potatoes, alfalfa, barley, and wheat. Three pulse crop species were intercropped with barley: lentil, pea, and chickpea. Barley was chosen because of its importance to the producers in Idaho, which has been the United States' leading barley producer since 2013. In 2020, Idaho producers harvested 500,000 acres of barley, representing 33.3% of the nation's supply (National Agricultural Statistics Service, 2020). Idaho is also one of the nation's leading producers of lentils and dry peas, with production concentrated in northern parts of the state and in the Palouse (Koong et al., 2020).

Two water regimes were utilized because water availability as both precipitation and irrigation is a key consideration for agricultural production statewide. Seasonal drought frequently occurs, especially in southern Idaho where precipitation timing does not coincide with critical stages of crop development. Most production in southern Idaho is reliant on irrigation, but this may also become increasingly uncertain. Persistent drought conditions in recent years have meant that irrigation water is often shut off early in the season, negatively impacting yields.

Water use in Idaho, as in much of the western US, is based on the date water rights were established (priority date). When there is not enough water to fulfill all the water rights, the most senior (oldest) water rights holders are permitted to satisfy their rights first. Water use moves in order down the priority list, meaning that more junior (recent) water rights holders will be left without water when the supply runs out. Further, while most barley production in Idaho is irrigated, most pulse production is dryland and relies on precipitation. Drought conditions have historically impacted Idaho for at least

one year each decade; more recently, county drought declarations occurred for 14 of the 18 years between 2000-2017 (“Economic Impacts of Climate Change on Agriculture in Idaho”, 2021). Accordingly, it is essential to estimate pulse crop water use and evaluate their production in response to drought stress as well as their impact on barley production to inform what regions and types of production practices may be suitable for intercropping.

The entire project will span four growing seasons, comparing barley/pulse intercropping with the practice of rotating barley and pulses every other growing season. Research from the first growing season (2020) is presented here. As a result of pathogens, chickpea was not viable during this growing season, so only lentil and pea responses are considered and discussed.

The aim of this work is a deeper understanding of the influence of intercropping on soil health, as well as the mechanisms behind those changes. Specific challenges arise when quantifying soil health related to soil organic matter retention and buildup, belowground soil microbial communities, and crop growth responses. Part of the difficulty can be attributed to parsing the “signal to noise” in field research. The bulk soil is a legacy of fertilization, crops, roots, microbial communities, etc., and it is difficult to quantify a treatment response at the time scale of a crop rotation. Thus, sophisticated methods that quantify carbon and nitrogen accumulation need to be coupled with more integrative measures to achieve a better understanding of soil health, soil microbial shifts, and plant physiological responses.

Integrative measures: Chapter 2

Chapter 2 examines the difference in soil health metrics across the growing season, integrating rapid and dynamic short-term fluxes into net changes. Shifts in soil C, N, and microbial communities are considered along with measurements of soil moisture and root biomass. This research goes beyond the surface by taking samples to a depth of 60 cm for roots and 90 cm for soil nutrient analysis, providing insights into soil health throughout the soil profile. The depth dimension is key for understanding agricultural systems as more than just plants and the soil surface.

Roots extend deep into the soil and are the physical actors driving the observed changes in many soil health measures. For example, they are the conduit through which N is taken up from soil solution (and soil gas, in the case of pulses), and C-rich root exudates are deposited. These exudates are crucial for microbial communities and influence soil structure by contributing to the formation of aggregates (Bronick & Lal, 2005; Huang et al., 2014). Therefore, sampling to depth allows us a more complete picture of the health of the soil that the crops are utilizing for growth.

Results from the first growing season are presented in chapter 2 and reflect relatively short-term changes as the system adjusts to new treatments; the experimental field had previously been planted with barley. The effects of intercropping and water treatments are expected to magnify over the four-year period of the study.

Sophisticated methods: Chapter 3

Understanding the specifics of short-term fluxes allows a mechanistic understanding of the cumulative changes over the growing season reported in chapter 2. As such, chapter 3 details the utilization of a sophisticated dual-label (^{15}N and ^{13}C) stable isotope approach to trace the flow of nutrients through the system over several weeks. Stable isotope labels can be traced as they are incorporated into pulse crops from the atmosphere via photosynthesis and nitrogen fixation and allocated within the plant to fuel growth and productivity. By sampling soil microbial communities in the pulse crop rhizosphere and roots from neighboring barley plants, the release of these fixed nutrients into the soil, where they are available for uptake, can also be observed.

The certainty of labeled nutrients coming from the pulse crop is increased because of the novel approach to ^{15}N labeling used in this work. In many tracer studies, ^{15}N is commonly introduced in the form of a solution containing ammonia or nitrate applied to the soil (e.g. Avice, Ourry, Lemaire, & Boucaud, 1996; Pirhofer-Walzl et al., 2012; Xiao, Li, & Zhang, 2004). These forms of inorganic N are typically found in fertilizer because they are available to all crops. We used ^{15}N enriched N_2 gas as our tracer, which is a less common approach primarily due to the high cost of the label (Chalk, He, Peoples, & Chen, 2017). However, because N_2 is not available to barley for direct uptake due to its lack of the symbiotic relationship with nitrogen-fixing bacteria that characterize pulse crops, this method allows us to confirm pulse crops as the origin of N label detected in sampled plant or microbial pools. The isotopic labeling method is detailed further in chapter 3; a schematic illustration is provided in Figure 1.2.

The findings in both chapters 2 and 3 are contextualized by considering aboveground crop biomass yield, as productivity is undeniably a critical aspect of soil health. However, plant physiology and agronomy are not considered in detail for this thesis. The work of additional researchers on this project will consider these aspects in greater depth.

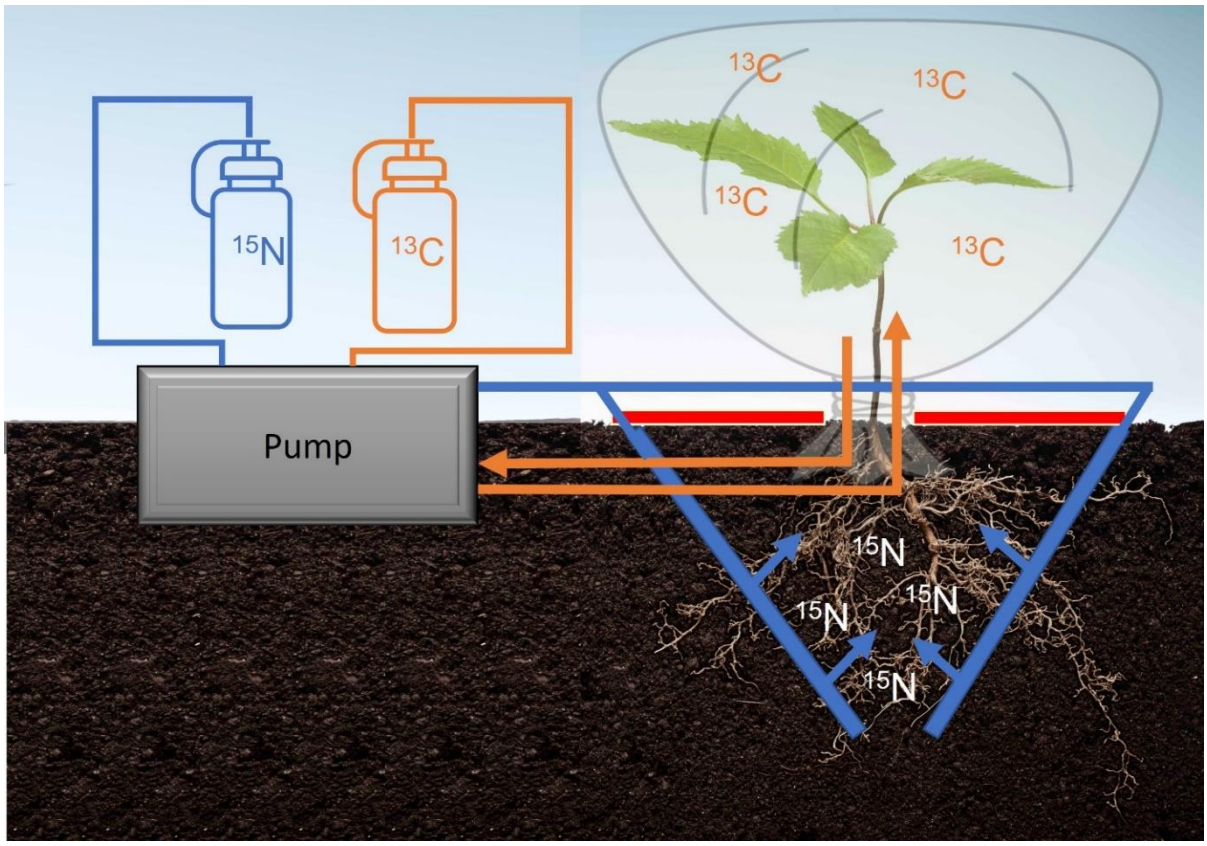

Figure 1.2: Schematic illustration of the dual-isotope labeling method. Blue lines represent the path of ^{15}N-N_2 from the source to the rhizosphere; orange lines represent the path of ^{13}C-CO_2 from the source to the canopy, enclosed within a transparent cuvette. Red lines illustrate PVC sheets placed on the soil surface to delay outgassing of N label and increase N uptake by pulse crop roots. The use of gaseous N label is a novel approach that validates the origin of traced N as the pulse crop.

Conclusion

In order to produce meaningful management recommendations for soil health in pulse production, baseline studies documenting direct changes are necessary. We will quantify the potential benefits of lentils, chickpeas, and dry peas in agronomic systems in terms of pulse and small grain crop physiology and soil health. This research directly addresses the priority of developing agronomic strategies to improve soil health through the incorporation of pulses in cropping systems. This research will close the knowledge gaps of pulse crops in terms of carbon and water cycling, impacts on soil health, and agronomic management and sustainability.

References

Arias, P.A., N. Bellouin, E. Coppola, R.G. Jones, G. Krinner, J. Marotzke, V. Naik, M.D. Palmer, G.-K. Plattner, J. Rogelj, M. Rojas, J. Sillmann, T. Storelvmo, P.W. Thorne, B. Trewin, K. Achuta Rao, B. Adhikary, R.P. Allan, K. Armour, G. Bala, R. Barimalala, S. Berger, J.G. Canadell, C. Cassou, A. Cherchi, W. Collins, W.D. Collins, S.L. Connors, S. Corti, F. Cruz, F.J. Dentener, C. Dereczynski, A. Di Luca, A. Diongue Niang, F.J. Doblas-Reyes, A. Dosio, H. Douville, F. Engelbrecht, V. Eyring, E. Fischer, P. Forster, B. Fox-Kemper, J.S. Fuglestvedt, J.C. Fyfe, N.P. Gillett, L. Goldfarb, I. Gorodetskaya, J.M. Gutierrez, R. Hamdi, E. Hawkins, H.T. Hewitt, P. Hope, A.S. Islam, C. Jones, D.S. Kaufman, R.E. Kopp, Y. Kosaka, J. Kossin, S. Krakovska, J.-Y. Lee, J. Li,T. Mauritsen,T.K. Maycock, M. Meinshausen, S.-K. Min, P.M.S. Monteiro,T. Ngo-Duc, F. Otto, I. Pinto, A. Pirani, K. Raghavan, R. Ranasinghe, A.C. Ruane, L. Ruiz, J.-B. Sallée, B.H. Samset, S. Sathyendranath, S.I. Seneviratne, A.A. Sörensson, S. Szopa, I. Takayabu, A.-M. Tréguier, B. van den Hurk, R. Vautard, K. von Schuckmann, S. Zaehle, X. Zhang, and K. Zickfeld, 2021: Technical Summary. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY,USA, pp. 33−144. doi:10.1017/9781009157896.002.

Avice, J. C., Ourry, A., Lemaire, G., & Boucaud, J. (1996). Nitrogen and carbon flows estimated by 15N and 13C pulse-chase labeling during regrowth of Alfalfa. *Plant Physiology*, *112*(1), 281–290. https://doi.org/10.1104/pp.112.1.281

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Chalk, P. M., He, J. Z., Peoples, M. B., & Chen, D. (2017). 15N2 as a tracer of biological N2 fixation: A 75-year retrospective. *Soil Biology and Biochemistry*, *106*, 36–50. https://doi.org/10.1016/j.soilbio.2016.12.010

Chen, G., Zhu, H., & Zhang, Y. (2003). Soil microbial activities and carbon and nitrogen fixation. *Research in Microbiology*, *154*(6), 393–398. https://doi.org/10.1016/S0923-2508(03)00082-2

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Economic Impacts of Climate Change on Agriculture in Idaho. (2018). *The Impact of Climate Change on Our Life: The Questions of Sustainability*, 179–195. https://doi.org/10.1007/978-981-10-7748-7_10

Goyal, R. K. (2004). Sensitivity of evapotranspiration to global warming: A case study of arid zone of Rajasthan (India). *Agricultural Water Management*, *69*(1), 1–11. https://doi.org/10.1016/j.agwat.2004.03.014

Huang, X., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., & Vivanco, J. M. (2014). *Rhizosphere interactions: root exudates, microbes, and microbial communities 1*. *275*(February), 267–275.

Intergovernmental Panel on Climate Change. (2014). Carbon and other biogeochemical cycles. In *Climate change 2013 – the physical science basis: Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change* (pp. 465–570). Cambridge University Press. https://doi.org/10.1017/CBO9781107415324.015

IPCC, 2022: Summary for Policymakers [H.-O. Pörtner, D.C. Roberts, E.S. Poloczanska, K. Mintenbeck, M. Tignor, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem (eds.)]. In: *Climate Change 2022: Impacts, Adaptation, and Vulnerability.* Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. In Press.

Keeling, C. D., Piper, S. C., Bacastow, R. B., Wahlen, M., Whorf, T. P., Heimann, M., & Meijer, H. A. (2005). Terrestrial Biosphere and Oceans from 1978 to 2000 : Observations and Carbon Cycle Implications. *A History of Atmospheric CO2 and Its Effects on Plants, Animals, and Ecosystems*, 83–113.

Koong, D., Benz, S., Johnson, B., Losh, D., Stebbins, E., & Reichert, J. (2020). 2020 Idaho Annual Statistical Bulletin. *USDA National Agricultural Statistics Service*.

Lal, R. (2014). Societal value of soil carbon. *Journal of Soil and Water Conservation*, *69*(6), 186A-192A. https://doi.org/10.2489/jswc.69.6.186A

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability (Switzerland)*, Vol. 7, pp. 5875–5895. https://doi.org/10.3390/su7055875

Linderholm, H. W. (2006). Growing season changes in the last century. *Agricultural and Forest Meteorology*, *137*(1–2), 1–14. https://doi.org/10.1016/j.agrformet.2006.03.006

National Agricultural Statistics Service, U. (2020). *Small Grains 2020 Summary*. (September).

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., & Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, *350*(1–2), 71–84. https://doi.org/10.1007/s11104-011-0882-z

Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*(1–2), 341–356. https://doi.org/10.1007/s11104-004-0907-y

Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N 2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(25), 9199–9204. https://doi.org/10.1073/pnas.1322434111

Stehfest, E., & Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems*, *74*, 207–228.

Strickland, M. S., & Rousk, J. (2010). Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Wang, S. L., Nehring, R., & Mosheim, R. (2018). Agricultural Productivity Growth in the United States: 1948-2015. *USDA, Economic Research Service, Amber Waves Magazine*.

Xiao, Y., Li, L., & Zhang, F. (2004). Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15N techniques. *Plant and Soil*, *262*(1–2), 45–54. https://doi.org/10.1023/B:PLSO.0000037019.34719.0d

Yang, T., Siddique, K. H. M., & Liu, K. (2020). Cropping systems in agriculture and their impact on soil health-A review. *Global Ecology and Conservation*, *23*, e01118. https://doi.org/10.1016/j.gecco.2020.e01118

Zhong, W., & Haigh, J. D. (2013). The greenhouse effect and carbon dioxide. *Weather*, *68*(4), 100–105. https://doi.org/10.1002/wea.2072

Chapter 2: Soil Health Metrics Under Barley-Pulse Intercropping

Introduction

Intercropping

Intercropping is the practice of growing two or more crops together on the same piece of land at the same time. Increasing biodiversity by growing more than one species mimics a more diverse natural ecosystem, and often confers benefits to the component crops that wouldn't be obtained alone. It also provides greater economic stability to producers against market changes or crop losses by diversifying their potential cash crops each season.

Intercropping pulses and cereal crops is common in some areas of the world because of their complementary resource acquisition strategy: deeper-rooted cereal crops may access water and nutrients from different depths in the soil profile than more shallow-rooted pulses (H. Hauggaard-Nielsen, Ambus, & Jensen, 2001). Additionally, the ability of pulses to fix N may reduce nitrogen competition to barley, alleviating N limitation with reduced inputs (H. Hauggaard-Nielsen et al., 2009; Henrik Hauggaard-Nielsen, Ambus, & Jensen, 2001; Rodriguez et al., 2020).

Several recent reviews have established the benefits of intercropping to crops, soil, and producers. These benefits include the reduction of pests and weeds, protection against pathogens, increased crop stability, lodging resistance, increased C inputs to the soil, more complete abiotic resource use, increased yields, decreased soil loss, and positive changes in the soil microbial community (Bedoussac et al., 2015; Glaze-Corcoran et al., 2020; Lian et al., 2019; Lithourgidis, Dordas, Damalas, & Vlachostergios, 2011). Another important benefit that intercropping may provide is increased resistance against yield loss under drought conditions through canopy shading decreasing soil evaporation or complementary water use patterns (Clermont-Dauphin et al., 2018; Renwick, Kimaro, Hafner, Rosenstock, & Gaudin, 2020; Walker & Ogindo, 2003).

Despite these benefits, intercropping is an agronomic strategy largely neglected by the large-scale monoculture growing practices that dominate modern agriculture (Brooker et al., 2015). Shifting large-scale production to intercropping from sole cropping practices would require changes such as the modification of existing machinery to efficiently plant and harvest intercropped fields. More research is needed so that producers can assess if adopting intercropping strategies is feasible and beneficial from both soil health and economic perspectives. Here, we focus specifically on the impact of intercropping on soil health.

Quantification of soil health

Several soil health evaluations exist (Soil Health Management Assessment Framework, Cornell Comprehensive Assessment of Soil) that measure various chemical, physical, and biological indicators of soil health. (Andrews, Karlen, & Cambardella, 2004; Moebius-Clune et al., 2016). Specific indicators may include organic matter content, bulk density, pH, electrical conductivity, plant-available N, soil nutrients, aggregation, and microbial community, depending on the goals and priorities of the assessment (Lehmann, Bossio, Kögel-Knabner, & Rillig, 2020). The key indicators measured in this research are soil moisture, root biomass, available C, inorganic N, and microbial communities. This combination of chemical and biological indicators provides a starting point for understanding our soils, and how they change under different crop and irrigation strategies.

Soil Nitrogen

A key part of the green revolution was the invention of the Haber-Bosch process, which allowed the synthesis of fertilizer that supplies plant-available forms of N to the soil. This radically altered soil fertility, and allowed farmers to grow crops in soils and at rates never before possible (Townsend & Howarth, 2010).

Today, the Haber-Bosch process supplies N at a rate nearly equal to that of biological and lightning N fixation, supporting food production for approximately 40% of the world population (Cherkasov, Ibhadon, & Fitzpatrick, 2015; Jenkinson, 2001). Accordingly, global fertilizer use has increased nearly tenfold from 11.3 Tg N $year^{-1}$ in 1961 to 110 Tg N for the 2020-2021 season (IFA – International Fertilizer Association, 2021; Lu & Tian, 2017). However, some research suggests that as little as 40% of the applied nitrogen is recovered in the harvested crops, meaning more fertilizer is lost than is taken up (Conant et al., 2013).

This loss represents a huge cost to both producers and the ecosystem. Excess fertilizer can pollute the soil, water, and air, harming the health of humans and other animals (Johnson et al., 2010; Savci, 2012). Further, fertilizer costs have been gradually rising since 2017 and spiked rapidly in late 2021 (McConnell, Liefert, Williams, & Boline, 2022). Prices are expected to remain high for the near future in response to supply chain disruptions caused by the Covid-19 pandemic and current global conflicts, threatening the economic future of many producers (Outlaw et al., 2022).

Clearly, there is a need to explore alternative sources of N that may reduce reliance on fertilizer. One common agronomic practice that is widely used to increase both soil N supply and crop diversity is including pulse crops (legumes) in crop rotations. Pulses are able to fix N through a symbiotic relationship with rhizobia bacteria on their roots which allows the conversion of abundant

atmospheric dinitrogen gas (N_2) to plant-available ammonium (NH_4^+). This source of biologically fixed nitrogen (BFN) decreases the reliance of pulse crops on existing soil N. When included in rotations, pulses increase soil N supply to subsequent crops as the residue left on the field is decomposed, releasing BFN to the soil.

There is growing evidence that pulse crops can also increase N availability on shorter time scales, even within the same growing season (Fustec, Lesuffleur, Mahieu, & Cliquet, 2009; Lesuffleur, Salon, Jeudy, & Cliquet, 2013; Pirhofer-Walzl et al., 2012). This transfer occurs through 3 methods: direct transfer facilitated by an arbuscular mycorrhizal fungi link, leachates or exudates (potentially translocated via fungal hyphae), and indirectly as nitrogen enters decomposition and mineralization processes in the soil (Høgh-Jensen, 2006; Thilakarathna, McElroy, Chapagain, Papadopoulos, & Raizada, 2016). This nitrogen transfer suggests growing pulse crops simultaneously with non-fixing crops (intercropping) may be a viable alternative to traditional rotation practices.

Objectives

This research aims to understand how intercropping impacts critical measures of soil health and productivity including organic carbon, plant-available nitrogen, root biomass, and soil microbial communities in an irrigated field in southern Idaho. Barley, an important cash crop in the region, was intercropped with two types of pulses: lentils and spring peas. Two species of pulse crops were included in order to assess if species selection matters when designing pulse intercrop systems.

Irrigation was applied at two levels to compare the impact of intercropping when water is not limited to when water is scarce. We hypothesized that intercropping would increase C inputs to the soil, N availability, and the abundance and diversity of microbial communities as compared to growing component crops alone. Further, we expected that the effects of water stress would be ameliorated under intercropping as compared to monocropping strategies.

Materials and Methods

Research Site

This research was conducted at the University of Idaho's Research and Extension Center in Aberdeen, Idaho. The experimental fields were planted first in spring of 2020, and soil samples were taken prior to planting and after harvesting in 2020 and 2021. The research will continue through the 2023 field season, with isotopic labeling campaigns carried out during the summer of 2020 and 2022.

The soil at this site is classified in the Xerollic Calciorthids family (UC Davis soilweb). Aberdeen receives an average of 23.2 cm of precipitation and 63.5 cm of snowfall annually and has an average annual temperature of 6.9°C (US climate data).

Experimental Design

Irrigation was applied at 2 levels: 100% (“full irrigation”) or 50% (“deficit irrigation”) of crop evapotranspiration. Crop evapotranspiration was calculated by multiplying reference evapotranspiration obtained from a nearby meteorological station (AgriMet Cooperative Agricultural Weather Network) by crop coefficients for each crop. Following common practice for southeastern Idaho, sprinklers were used to apply irrigation.

Crops consisted of two pulse crops (spring peas and lentils) and barley, planted either alone (monocropped) or in alternate rows of pulse/barley combination (intercrop). This resulted in a total of n = 5 unique crop mixes: barley only (BO), lentil only (LO), pea only (PO), intercropped lentil/barley (LB), and intercropped pea/barley (PB). Barley was selected because of its significance to Idaho agriculture; Idaho is the nation’s leading barley-producing state.

A split-plot design with N=4 replicate blocks was used to test combinations of irrigation and crop strategies at 4 soil depths. Phospholipid fatty acid (PLFA) samples for microbial analysis were taken from only the surface layer; all other samples were taken at 4 depths to 90cm. Each main plot was divided into 2 sub-plots for irrigation treatment, resulting in a total of n = 8 sub-plots. Within each sub-plot, crop mix treatments (n = 5) were randomly assigned for a total of 40 unique experimental units.

Soil samples for nutrient analysis were taken from each plot prior to planting in April 2020 (T0) and after harvesting in August 2020 (TF). Soil cores were obtained using a 3.8 cm diameter soil auger at 4 depth intervals: 0-15 cm (depth 1), 15-30 cm (depth 2), 30-60 cm (depth 3), and 60-90 cm (depth 4). Root samples were collected during TF soil sampling, using 2 mm sieves to separate roots from soil cores in the field. Root samples were taken at equidistant depth increments to a shallower depth than those of soil only: 0-15, 15-30, 30-45, and 45-60 cm.

Soil samples for phospholipid fatty acid (PLFA) analysis were taken in June 2020 (T0) and August 2020 (TF) using a small-diameter soil sampler to a depth of 8cm.

Experimental plots measured 3.05 meters wide by 6.1 meters long with 14 rows spaced 17.8 cm apart. In monocropped plots, all 14 rows were of the same crop, whereas in intercropped plots the rows alternated between barley and the pulse crop, with 7 rows of each in total. A 12.2 meter buffer of short-stature rye grass was planted between each main plot to minimize irrigation drifts.

Assays

Soil moisture was recorded using 5TM water content and temperature sensors (Decagon Devices, Inc.) installed at 30 and 60 cm. Recordings were taken hourly throughout the growing season using Campbell data loggers. Cumulative moisture aggregated from hourly data taken during the growing season (May 10 to August 7, 2020) was plotted.

At the end of the growing season, soil core samples were sieved through a 2mm screen in the field. The material that passed through the sieve was subsampled for nutrient (C, N) analysis. The portion that did not pass through the screen was retained; roots were later separated from soil aggregates and debris using a root washer. Washed samples were scanned using WinRHIZO image analysis to obtain root length and volume and oven dried for root biomass.

Soil samples were air-dried and homogenized using a SPEX 8000D Mixer/Mill ball mill prior to analysis. Active carbon was quantified using the potassium permanganate oxidizable carbon (POX-C) method (Culman, Freeman, & Snapp, 2012). Inorganic nitrogen (as NH_3 and NO_3^-) was quantified using KCl extraction and a Lachat Quikchem Flow Injection Analyzer (Hach USA).

To account for heterogeneity in soil nutrient status across depths and plots, initial soil POX-C and N measurements were subtracted from end-of-season measurements, and the change was expressed as a percent. Raw values of C and N change (mg/kg soil) are provided in supplemental tables 1 and 2.

PLFA soil samples were immediately freeze-dried until analysis following a modified Bligh-Dyer extraction method (Quideau et al., 2016). Extracted samples were analyzed using GC-FID (Agilent Technologies) and Sherlock software (MIDI, Inc) to identify fatty acids and further categorize them by functional group (Supplemental Table 1).

Analysis

Changes in microbial biomass C (nM g^{-1} soil) across the growing season were calculated by subtracting averages for each treatment at T0 from final biomass for each microbial group; the standard error around these values was propagated (Figure 2.4). Tukey's Honest Significant Difference test (TukeyHSD() in R) was used to identify treatments with significantly different responses across all functional groups.

Considering all identified PLFAs rather than only those which are assigned to a functional group allows a broader view of the total soil microbial community. A Bray-Curtis distance matrix was calculated for each sample and used as the response variable in a permutational manova (vegdist() and adonis2(); 'vegan' package in R) to assess if end-of-season samples were significantly different

from initial samples. Principle coordinates analysis was conducted on TF samples (cmdscale() in R) to visualize the community differences represented in the calculated distance matrix (Figure 2.6).

Partial least squares regression was performed to examine correlations of total microbial biomass (nanomoles of C/gram of soil) at TF with water and crop treatments, as well as inorganic N and available C at all depths, root mass, pH, crop biomass yield, and C:N ratios at all depths (plsreg1(); plsdepot package in R). This regression is most appropriate in this instance because it considers the large number of predictor variables without assuming they are fixed. Where plot-specific data for any explanatory variables were missing, averages for that crop and water treatment were used in their place, so that observations were not dropped (n=39).

Results

Soil Moisture

The effect of both crop and irrigation treatments on cumulative volumetric water content were significant at both depths ($p<0.001$) (Figure 2.1). The aggregation of small differences in daily measurements between crop strategies within each irrigation treatment is more clearly visualized when cumulative soil moisture is considered, rather than daily averages.

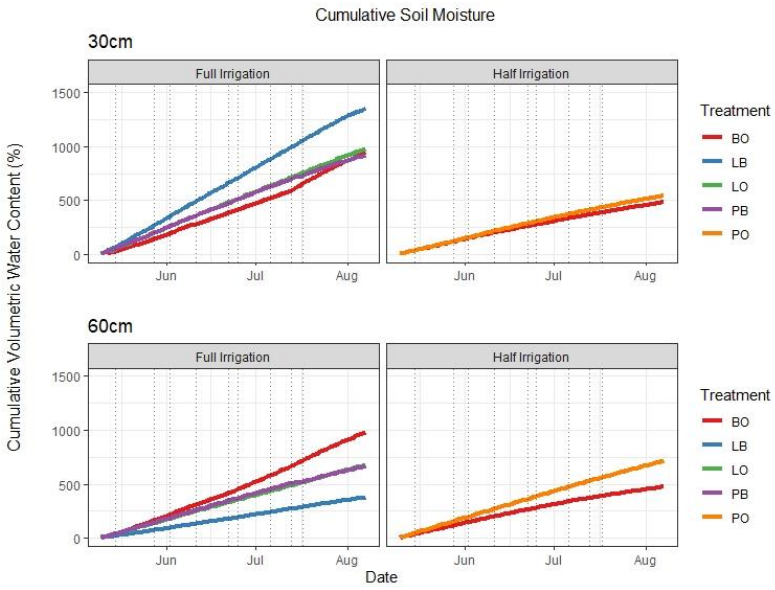

Figure 2.1: Cumulative soil moisture at 30cm (top panel) and 60cm (bottom panel). BO = barley only, LO = lentil only, LB = lentil/barley, PO = pea only, PB = pea/barley. BO plots at 60cm had significantly higher water content than all other crop strategies under full irrigation, but lower under deficit irrigation, suggesting barley grew deeper roots in response to water limitation. A similar pattern is seen for LB within full irrigation.

Within the full irrigation treatment, the lentil/barley (LB) treatment was significantly different from all other crop treatments at both depths; at 30cm the water content was highest, and at 60cm it was the lowest of all treatments. At 60cm, barley only (BO) had significantly higher water content than all other treatments.

Within deficit irrigation, soil moisture data were only collected under two crop treatments: BO and PO. At both depths, BO had significantly less water content than PO; this difference was greater at 60cm.

Root Mass

There is not a significant difference in total root mass by location between irrigation or crop strategies, though the interaction of crop and irrigation strategy was significant ($p<0.05$).

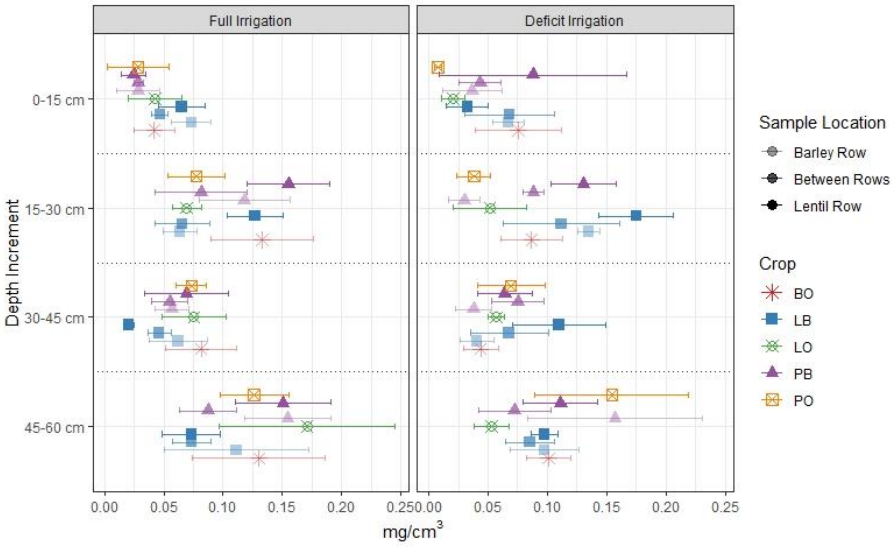

Figure 2.2: Mass of roots obtained from soil cores at depth increments to 60cm. BO = barley only, LO = lentil only, LB = lentil/barley, PO = pea only, PB = pea/barley. Sample location indicates the crop row from which the sample was obtained; root mass is assumed to represent the crop from that row and to be mixed for "between row" sample location.

For both irrigation strategies, the greatest root mass for BO plots occurs between 0-15 cm and 30-45 cm, whereas pulse-only crop treatments tend to have greater root mass near the surface and decreasing mass with depth (Figure 2.2).

Within deficit irrigation, crop strategies did have a significant effect on root mass distribution. This is most apparent when comparing lentil root mass between LO and LB crop treatments; LB plots had more root mass at every depth than LO plots.

LO roots have a relatively consistent pattern across both irrigation treatments, with the greatest root mass at 0-15 cm and subsequent mass decreases with depth. In contrast, lentil roots from LB plots have the greatest mass from 30-45 cm.

Soil Carbon and Nitrogen

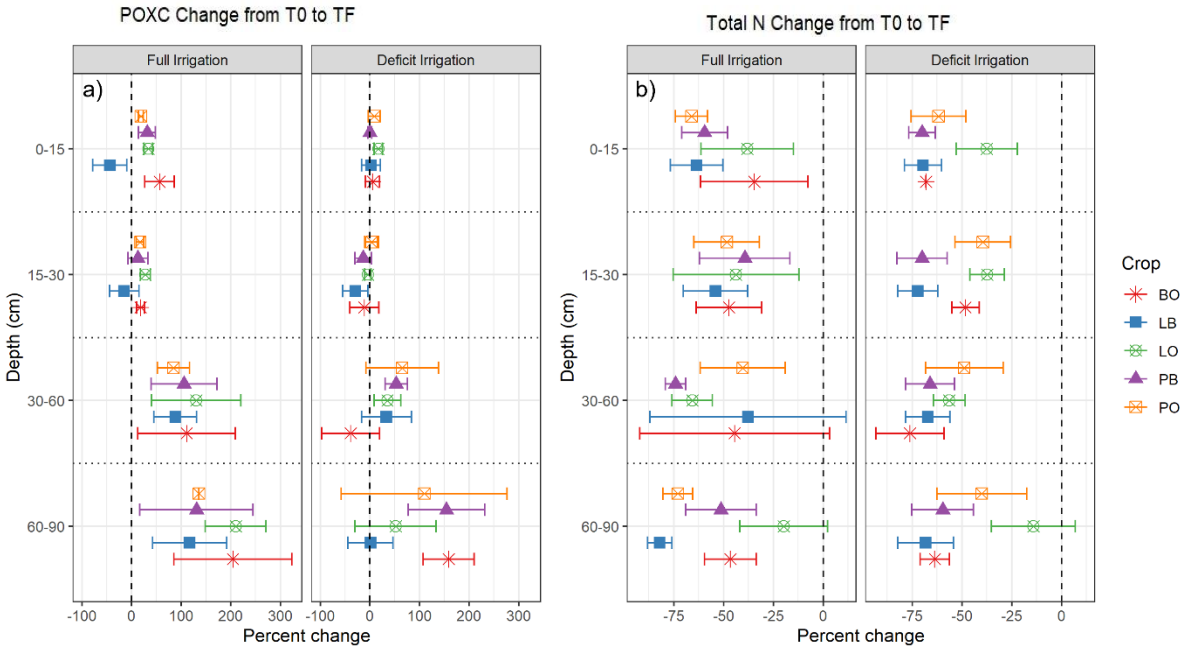

Figure 2.3: Percent change in soil C (left) and N (right) across the growing season. BO = barley only, LO = lentil only, LB = lentil/barley, PO = pea only, PB = pea/barley. Accumulation of nutrients at depth, particularly under full irrigation could indicate translocation due to leaching. Under deficit irrigation, crop treatments including pea contributed the most C to the soil and monocropped pulses had the smallest decreases in soil N, indicating a reliance on BFN for some portion of pulse N demand.

Carbon

Potassium permanganate oxidizable C (POX-C) is a widely-used metric in soil health tests to represent the biologically active pool of carbon (Norris et al., 2020; Wade et al., 2020). Carbon additions to the soil during the growing season can primarily be attributed to rhizodeposition, whereas plant residue and microbial necromass contribute to changes over a longer time period (Franzluebbers, Hons, & Zuberer, 1995). This carbon is available to microbial communities and is linked to soil health properties such as nutrient storage, water holding capacity, and aggregate stability as well as crop yield (Bennett, Mele, Annett, & Kasel, 2010; Cardoso et al., 2013).

Neither crop nor irrigation strategy had a significant effect on active carbon levels (Figure 2.3, left panel). The increase in C across the growing season at 60-90 cm was significantly different from the changes at all other depths.

The percent change in POX-C input to the soil increases with depth, but the magnitude of the accrual in C (mg/kg soil) decreases. For example, carbon accrual in fully irrigated BO plots was approximately twice as large at 0-15 cm as compared to 60-90 cm, but the percent change in C was nearly 6x smaller (Supplemental Table 2.1).

There is almost no change in C at any depth for lentil/barley intercropping under deficit irrigation. Under full irrigation, C slightly decreases from 0-30 cm, but increases deeper in the soil profile. Under deficit irrigation, PB soil C matches that of PO at 30-60 cm and of BO at 60-90 cm.

Nitrogen

Extraction of soils with 2M KCl allows for the quantification of inorganic N species (NO_3^- and NH_4^+), available for plant uptake (Keeney & Nelson, 1983). Understanding soil N changes across the growing season is a key tool for evaluating how intercropping barley with pulse crops influences N dynamics, and if pulse intercropping can alleviate N limitation or promote more complete use of soil N. Experimental plots were not fertilized, so increases in N observed at 60-90 cm indicate either net deposition to the soil from pulse crops or relocation of existing soil N via leaching.

As with C, the only significant effect on percent of inorganic N change is depth in the soil profile; specifically, the difference between 60-90 cm and the other depths is significant (Figure 2.3, right panel). For both irrigation treatments, the only increases in soil N were observed at 60-90 cm in crop treatments that included pulses.

Under deficit irrigation at every depth, the decrease in soil N as both percent and absolute change across the growing season is smaller for the monocropped pulse plots than for the intercropped plots. This pattern is especially apparent at depths 2 and 4, where monocrop and intercrop plots are clearly separated, with BO intermediate between the two.

When crop treatments are grouped by strategy (monocropped barley, monocropped pulse, intercropped barley/pulse), the effect on N change is significant ($p<.05$) for deficit irrigation, though not for full irrigation. Under deficit irrigation, intercropping produces a response of lower soil N at the end of the growing season compared to monocropping that is not observed under full irrigation conditions.

Phospholipid Fatty Acids

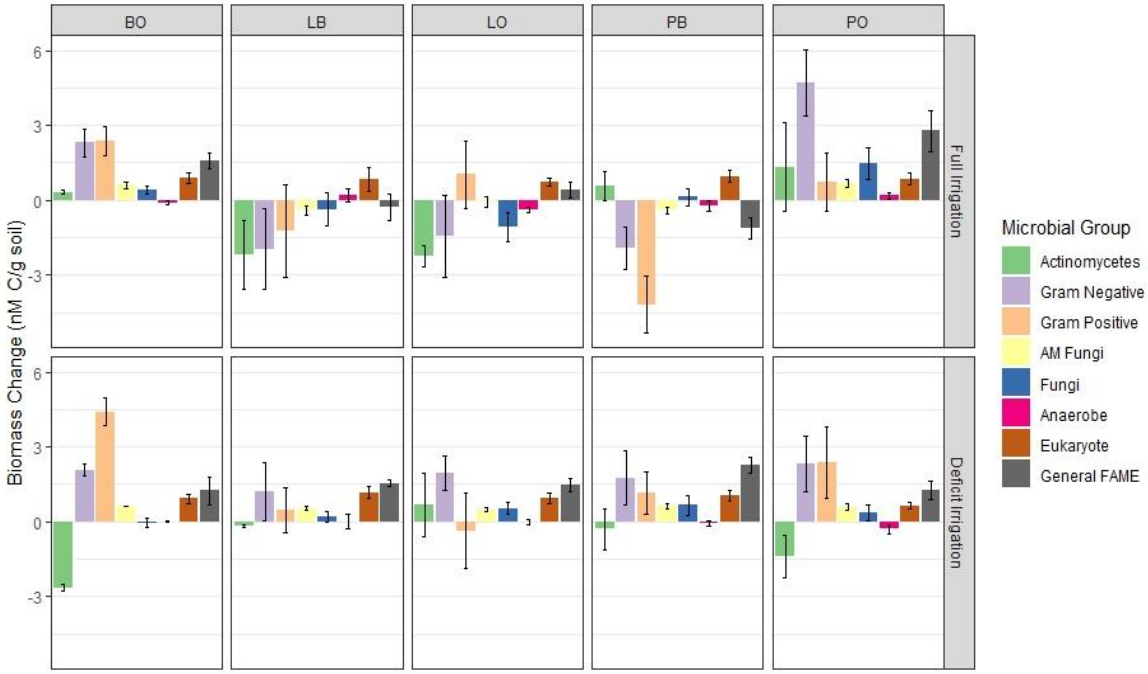

Figure 2.4: Change in microbial biomass C across the growing season, separated by microbial groups. BO = barley only, LB = lentil/barley, LO = lentil only, PB = pea/barley, PO = pea only. Groups are arranged on the x-axis by broader classification as bacteria (actinomycetes, gram-negative, and gram-positive groups) or fungi (AM fungi and fungi) for easier interpretation. Full irrigation PO is the only treatment with no decreases in biomass for any microbial group; the interaction of pea and barley when intercropped (PB) elicits bacterial decreases not exhibited in either component crop (BO, PO) grown alone.

All treatments had both decreases and increases within various microbial groups except for full irrigation PO, where the biomass of all microbial groups increased (Figure 2.4). Full irrigation PO microbial biomass change was significantly different ($p<0.05$) from full irrigation LO, LB, and PB; the latter three groups were also the only treatments with net decreases in microbial biomass across the growing season.Under deficit irrigation microbial biomass generally increased, except for bacterial groups in some crop treatments and anaerobes in PO. This mixed response aligns with other

reports of bacterial biomass in response to water limitation, with reports of increases, decreases, and no change all reported by various researchers (Naylor & Coleman-Derr, 2018). These changes may arise because of the effect on drought on fine root turnover and the quality and amount of organic C inputs from root exudates, which in turn impact decomposition (Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2014). Fungal group biomass in deficit irrigation treatments exclusively increased, whereas under full irrigation the fungal response was mixed across crop treatments.

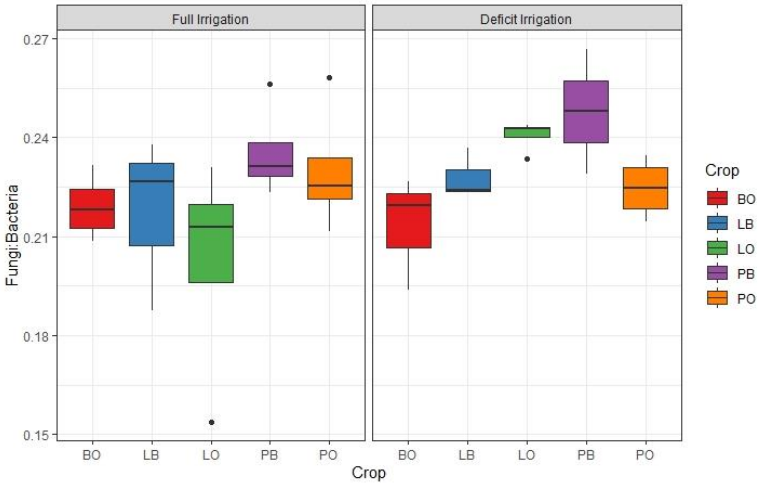

Figure 2.5: Fungi:bacteria ratio (F:B) at TF for each crop and water treatment. BO = barley only, LB = lentil/barley, LO = lentil only, PB = pea/barley, PO = pea only. F:B was generally higher in deficit irrigation than full irrigation, indicating fungal dominance in water-limited conditions.

The ratio of fungi to bacteria (F:B) in soil is commonly used as a shorthand microbial indicator of soil response to management changes and C sequestration potential (Malik et al., 2016). F:B was calculated by aggregating the biomass of fungi (AM fungi and fungi) and bacteria (actinomycete, gram-negative, and gram-positive) groups (Figure 2.5). Differences between full and deficit irrigation were not significant at $p<0.05$, but a general increase in deficit irrigation F:B was observed. Fungi are typically considered to be more drought-tolerant than bacteria and are more competitive (produce more biomass) than bacteria in conditions where water is limiting, increasing F:B (Strickland & Rousk, 2010; Yuste et al., 2011).

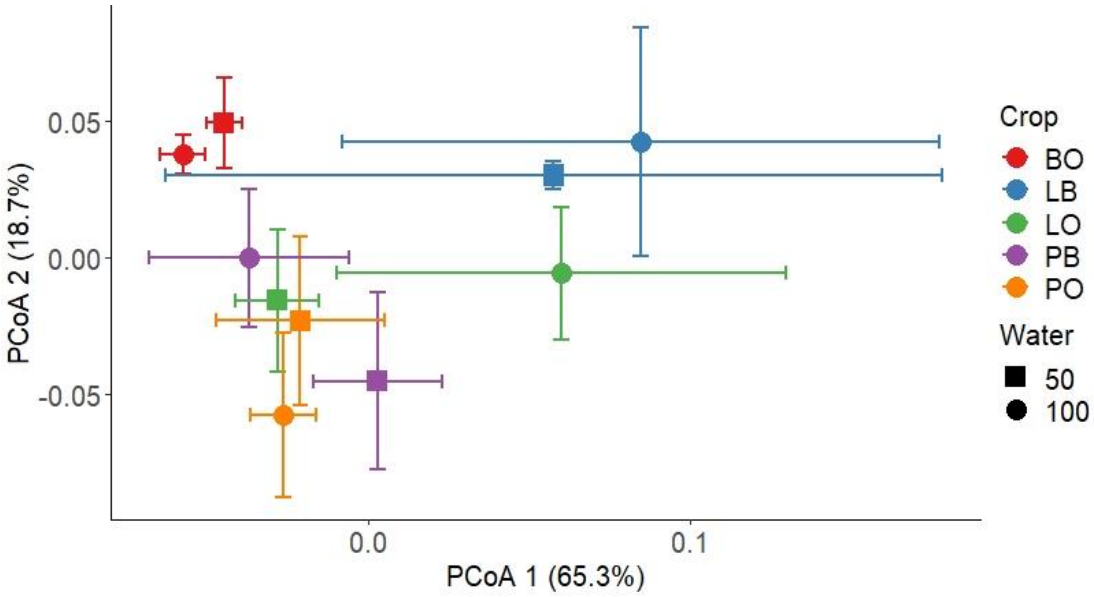

Figure 2.6: Principal coordinates analysis of microbial communities for each crop and water treatment at the end of the growing season (TF). Axes 1 and 2 appear to be explained by water and crop treatment, respectively. Crop treatments including lentil (LO, LB) are more dissimilar to other treatments, indicating a shift in microbial community composition when this crop is included. Greater distance between points reflects greater dissimilarity in communities; the relatively tight clustering of BO communities suggests similar microbial communities between water strategies for this crop.

Principal coordinates analysis (PCoA) was used to represent the microbial community structure under each combination of crop and water treatment (Figure 2.6). In this ordination, samples (n = 4) from each combination of treatments are collapsed into centroids representing the multivariate means with standard error bars on each axis. Each point represents all samples from a combination of treatments where the variables (PLFAs) are summarized in reduced dimensions while preserving the distance between samples from a dissimilarity matrix. The percentages on the axes represent the amount of variance among the samples that is explained by the axis. Points that are clustered more closely together indicate communities that are more similar.

PLFA samples at the end of the growing season (TF) were significantly different from paired samples taken prior to planting (T0), indicating changes across the growing season were driven by the experimental treatments. LB treatments under both irrigation and full irrigation LO stand out on axis 1, meaning crop treatments that included lentil had the most distinct communities between irrigation strategies. The proximity of BO communities under both irrigation treatments away from other treatments indicates microbial communities of BO plots were similar to each other, but distinct from all other crop strategies.

Plotting all measured variables in a correlation circle based on a partial least squares (PLS) regression model allows the visualization of the relationship between all variables and helps to identify what soil factors are most strongly correlated with total microbial biomass (Figure 2.7). In this ordination, positively correlated variables are grouped together, and variables negatively correlated with the response variable (microbial biomass) appear in opposing quadrants.

The two treatment effects (water and crop) are somewhat correlated with microbial biomass, but the strongest predictor variable is carbon at soil depth 3 (C3, 30-45 cm). Considering PLFA samples were taken from a depth of 0-15 cm, the correlation of deeper C with surface microbial biomass could be explained by C leaching from shallow origins and accumulating at greater depth in the profile (Figure 2.3, left panel).

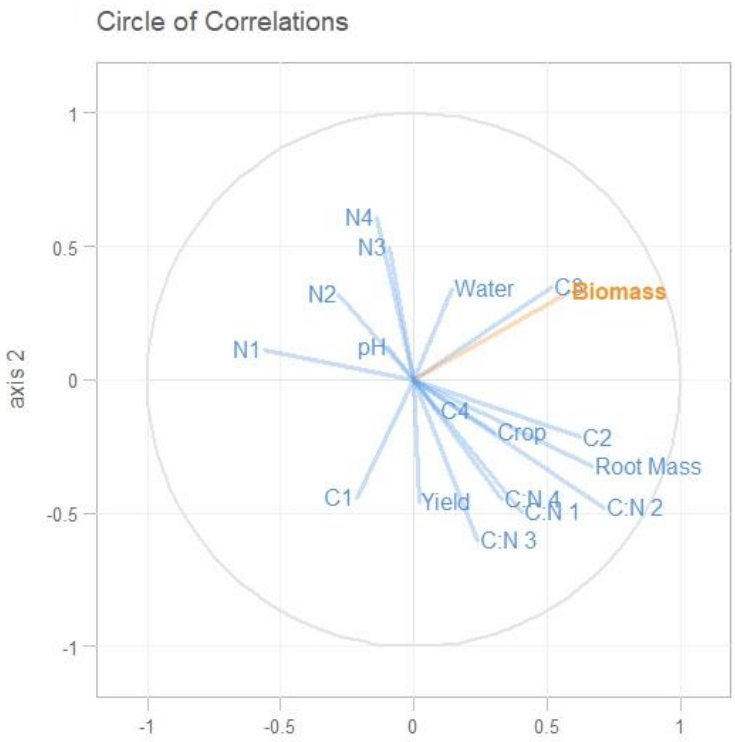

Figure 2.7: Circle of correlations for measured variables/treatments and microbial biomass (nM C/g soil). Carbon at depth 3 was most correlated with microbial biomass; crop and water treatments are weakly correlated.

Conversely, C1 is strongly negatively correlated with microbial biomass. Soil inorganic N (N) at all depths group in the same quadrant (top left), C:N ratios at all depths group similarly in the opposite quadrant. The total variation explained by this model (R^2) is 0.596. The explained variance of each variable by PLS components (n =17) is reported in Supplemental Table 2.6.

Irrigation	Crop	Intercrop Biomass	Sole crop Biomass	Percent of Sole Crop Biomass
Full	Barley (with lentil)	1532.4	2387.5	64.18*
Full	Barley (with pea)	1660.2	2387.5	69.54*
Full	Lentil	235.2	1504.2	15.64*
Full	Pea	273.1	1622.2	16.84*
Deficit	Barley (with lentil)	1343.1	1625.0	82.65
Deficit	Barley (with pea)	1414.8	1625.0	87.06
Deficit	Lentil	154.9	1287.5	12.03*
Deficit	Pea	171.3	1481.9	11.56*

*Table 2.1: Biomass yield comparison between sole crop and intercrop strategies for both irrigation treatments. Barley biomass decreases were significant under full irrigation but were not significant under deficit irrigation (significance at $p<0.05$ indicated with *). Deficit irrigation induced greater biomass decreases for pulse crops than full irrigation, but smaller biomass decreases for barley indicating that in water-stressed conditions, barley is more competitive than pulse crops for soil resources.*

Discussion

In this study, we intercropped barley with pulses as an approach to increase agroecosystem diversity and understand the resulting impacts on soil health. Specifically, we focused on changes in soil N, organic C, root mass, and microbial communities as key indicators of soil health under two irrigation strategies. We found that there are interesting feedbacks between plant species, nutrients, and microbial communities that are largely driven by water availability at this site. The distinct nature of pea and lentil pulse crops is also demonstrated in each of these aspects, establishing that crop choice is important in intercropping systems.

Full Irrigation

During the growing season, full irrigation plots received 283 mm of irrigation; twice as much as deficit irrigation plots (142mm). This treatment was designed to simulate a growing scenario where water availability was not a limiting factor for crop growth and understand barley-pulse interactions under “non-stressed” conditions.

Irrigation water combined with relatively sandy soil textures mean that translocation of soluble C and N compounds throughout the soil profile from leaching is likely to have obscured depth-based changes in nutrient status under full irrigation. As such, subsurface nutrients should not be considered to have necessarily originated where they are detected and conjectures about the cause of changes must be made cautiously.

For example, decreases in POX-C across the growing season could be a result of microbial activity mineralizing C or leaching to lower depths in the profile (Manninen, Soinne, Lemola, Hoikkala, & Turtola, 2018). Coinciding patterns of decreases in POX-C at shallower (>30 cm) soil depths and increases at depths >30 cm (Figure 2.3, left panel) favor leaching and deposition as the driving factor.

Similar increases in soil N at a depth >60 cm in crop strategies that include pulses were also observed, reinforcing the idea of depth transport mechanisms driving patterns in nutrients through the soil profile. Relatively small C increases in deficit irrigation LB plots, where less leaching would be expected to occur, further support the significance of leaching.

Deficit Irrigation

Roots:

Water stress appears to induce a deeper rooting strategy for barley, as evidenced by greater root mass in monocropped barley (BO) at the 45-60cm depth under deficit irrigation than full irrigation (0.075g cm^{-3} as compared to 0.042g cm^{-3}) (Figure 2.2). Lower cumulative soil moisture at 60cm for BO under deficit irrigation than PO further supports barley's reliance on water deeper in the soil profile (Figure 2.1).

Similarly, water competition with the companion pulse may have driven barley roots in intercropping treatments deeper than when barley is grown alone, explaining the reversal of LB and BO soil moisture trends at 30cm compared to 60cm (Figure 2.1). Some research suggests that intercropping promotes more complete use of water in the soil profile, as deeper-rooted barley crops access soil water from different soil horizons than shallow-rooted pulses, creating water resource "niches" for each crop (Li et al., 2006; Walker & Ogindo, 2003). Our research supported this, as total soil profile moisture (30 and 60cm) was lower for LB and PB than BO, indicating that more soil water was taken up under intercropping as compared to when barley was grown alone (Supplemental Table 2.3).

In addition to water, crops also compete for critical nutrients such as soil N. Within deficit irrigation, where leaching is not expected to obscure depth-specific measurements, soil N was used more completely (greater absolute and percent decreases) in intercropped plots (LB, PB) than monocropped pulse plots (LO, PO) at every depth (Figure 2.3, Supplemental Table 2.2).

Carbon

Within deficit irrigation, greater C increases in crop treatments including pea (PO, PB) than those including lentil indicate C inputs differ among pulse crops. Stable isotope labeling results support these findings; a greater amount of pulse crop-derived C was detected in soil microbial communities with pea as compared to lentil (chapter 3).

Increasing soil C is critical for supporting the health and productivity of soils as well as the ecosystems they are part of (Lal, 2014; Stockmann et al., 2013). The increases in soil C observed in treatments including pea reinforce that the type of pulse crop (pea or lentil) included in intercropping

rotation matters. Management considerations such as irrigation and field-specific factors like soil texture will also play a role in the magnitude and location of soil C.

Microbial community and water

Profiling the microbial community through phospholipid fatty acid (PLFA) analysis is one method of testing soil health and response to management changes (Bossio, Scow, Gunapala, & Graham, 1998; Mann, Lynch, Fillmore, & Mills, 2019). Services such as nutrient cycling, pathogen and pest defense, aggregation formation, organic matter stabilization and water availability are provided by microbes, so understanding the response of microbial communities to management changes is an essential tool for understanding soil health (Lehman et al., 2015; C. Li, Cano, Acosta-Martinez, Veum, & Moore-Kucera, 2020). Various authors have proposed methods of grouping and interpreting PLFA communities; the relative abundance of fatty acids has been linked to soil properties including carbon availability, water content, pH, and compaction (Bossio & Scow, 1998; Rousk, Brookes, & Bååth, 2010; Shestak & Busse, 2005; Zelles, 1999).

Soil organic C in particular is known to have a strong influence on microbial community biomass, as it is a source of energy for microbial growth (Jiang-shan, Jian-fen, Guang-shui, & Wei, 2005; Wardle, 1998) (Figure 2.7). Additionally, soil C:N may be a measure of how available nutrients are for microbial growth; since soil microbes typically have well-constrained C:N ratios that fall between 8:1 and 12:1, soil C:N ratios that match this ratio will be more available to microbes (Cleveland & Liptzin, 2007; Wan et al., 2015). The extremely low correlation between microbial biomass and soil C:N (Figure 2.7) suggest that the C:N ratio of the soil in this study likely does not fall within the range most accessible to microbes.

Considering microbial biomass change, irrigation appears to have a greater impact on microbial communities than crop strategy; under deficit irrigation, significant changes between monocropping and intercropping strategies were not observed. Under full irrigation, PO had significant increases in microbial biomass compared to all other crop treatments including a pulse crop. This further suggests that growing peas may increase soil microbial biomass. However, when intercropped with barley, decreases in biomass were observed, indicating the interaction of pea and barley does not favor microbial growth.

The lack of significant changes in F:B between treatments indicates intercropping does not create water stress that is reflected in the soil microbial community. Water stress was implied by slightly higher F:B observed for most crop treatments under deficit irrigation. These changes may not have been significant due to the relatively short interval between sampling (52 days). More robust changes

in F:B may occur over subsequent growing seasons as fungi are more competitive under persistent water stress better than bacteria. This ratio is a response to soil moisture, but increased fungal populations serve important functions contributing to soil health, including protection against pathogens and drought, N fixation and nutrient transfer, and decomposition and stabilization of organic matter (Frac, Hannula, Belka, & Jędryczka, 2018).

When treatment communities are aggregated (PCoA; Figure 2.6), water treatment appears to explain spread on axis 1 (64% of variability) and crop treatment explains spread on axis 2 (18.4% of variability). This further supports irrigation having a greater impact on microbial communities than crop strategy. Intercropping lentils (LB) resulted in the greatest community composition shift as compared to BO. The unique response of treatments including lentils and those including peas highlights that pulse crops do not have uniform effects on soil microbial communities. Understanding these effects is critical for soil health and resilience to disturbance, which has been linked to microbial diversity (Jiao, Wang, Wei, Chen, & Lu, 2019).

Species selection and N fixation

Barley is more competitive for soil N than pulse crops, likely due to deeper and faster-growing roots, and has also been shown to increase N acquisition in response to competition from intercropping (H. Hauggaard-Nielsen et al., 2009; Jensen, 1996). Obtaining soil N is less energetically costly than biological N fixation, leading to decreases in soil N across the growing season for all crop treatments, even monocropped pulses (Figure 2.3, right panel)
More complete use of soil N under intercropping has previously been observed, and is attributed to the stimulation of barley N uptake by including less competitive pulse crops, reducing overall N competition to barley in the soil (Corre-Hellou, Fustec, & Crozat, 2006). This dynamic is enhanced as BFN obtained by pulse crops reduces their reliance on soil N (H. Hauggaard-Nielsen et al., 2001).

However, BFN is not a uniform process across all species, so not all pulse crops can be considered interchangeable in terms of N dynamics. Plants with symbiotic N-fixation abilities have broadly been classified as “obligate” or “facultative” N-fixers, based on changes in the amount of N fixed per unit of biomass as soil N status changes (Menge, Levin, & Hedin, 2009). Facultative N_2 fixers can respond to increased soil N availability by downregulating fixation, obtaining just enough N to meet their own needs without wasting energy on excess fixation. Obligate N_2 fixers do not change the amount of N fixation regardless of soil nutrient status, which can enhance N availability to companion crops as excess N is released to the soil (Menge, Wolf, & Funk, 2015). Previous work has demonstrated both obligate and facultative N-fixation strategies in various herbaceous legumes

(Dovrat, Bakhshian, Masci, & Sheffer, 2020; Drake, 2011). Specifically, peas have previously been shown to increased root nodulation and subsequent N_2 fixation in intercropping as compared to sole cropping, indicating a facultative fixation strategy (Chapagain & Riseman, 2014).

Considering deficit irrigation plots with greater depth-based nutrient integrity, lentils and peas do not produce identical responses, suggesting that their N-fixation strategies vary. Monocropped peas (PO) take up more soil N than LO at all depths, indicating a greater reliance on soil N compared to BFN for peas than lentils. However, under intercropping, N uptake in PB plots is very similar to that in LB plots between 15 and 60cm. This could indicate that pea has a degree of facultative control over BFN; increased nodulation on intercropped pea roots as compared to sole cropped pea roots has been observed (Chapagain & Riseman, 2014). When grown alone, pea will rely on the more energetically inexpensive N from the soil, but when competition for this N is increased through intercropping with barley, it can upregulate BNF to meet its N demand.

These results alone are not conclusive—stable isotope labeling results suggest obligate fixation strategies, with transfer of BFN to neighboring barley observed in both pea and lentil intercropping systems during a limited period (<3 weeks) of the growing season (see chapter 3). Results reported here represent the culmination of these short-term changes over the entirety of the growing season, however, giving a more complete look at net N use. More detailed analysis of plant N content (as in Dovrat, Bakhshian, Masci, & Sheffer, 2020) would be required to further elucidate the degree of flexibility in the N-fixation strategy of peas and lentils.

Intercropping pulse species with barley resulted in distinct changes in soil health measures. Organic C deposition and microbial community diversity were both increased by intercropping, particularly when lentil was the pulse species. Intercropping also resulted in greater uptake of both soil water and N than monocropping, likely due to complementary differences in root depth and N acquisition strategies that reduced overall competition to each crop.

Changes in soil health are further reflected in biomass yields, which responded strongly to the irrigation treatment. Overall, the biomass of both component crops was reduced in intercropping, though the reduction in barley was only significant ($p<0.05$) within the full irrigation treatment. The average reduction for each crop was 24.1% for barley and 86% for pulse crops (Table 2.1), suggesting barley outcompeted pulse crops for resources when grown together. Under deficit irrigation, the effects of this competitive disparity were greater: barley biomass decreased slightly (average of 15.1%) and pulse biomass decreased significantly (average of 88.2%). This suggests that the effects

of intercropping are highly dependent on water availability, which must be considered by producers when assessing the suitability of intercropping for their specific growing conditions.

Conclusion

This study sought to understand if intercropping barley with pulse crops could improve soil health through impacts on soil properties like plant-available N, organic C inputs, and microbial communities. These properties were measured under both well-watered and water-stressed conditions to further evaluate if intercropping can mitigate the effects of water limitation. This is particularly important to understand for dryland growers that rely on precipitation, but also for irrigated sites that may have unreliable water supply due to junior water rights.

We hypothesized that pulse intercropping would result in more complete use of soil water and nitrogen, increase C additions to the soil, and increase belowground microbial biomass and diversity as compared to sole cropping. Our results support these hypotheses and indicate that pea and lentil pulse crops do not have identical impacts on soil health. Overall, intercropping reduced competition for soil water and N for barley, resulting in less impacted biomass yields for barley; pulse crop biomass yield was significantly reduced in intercropping.

The application of these results is limited by the short time scale of the work. As this is the first growing season with our treatments applied, legacy effects of past management may obscure responses caused by crop or irrigation treatments. Results from the following 3 years of this work will help to clarify the longer-term changes caused by intercropping and irrigation strategies. Further, since monocropped barley plots are rotated with monocropped pulse in alternate years, these future results will allow direct comparison between barley/pulse rotations and barley/pulse intercropping at the same site. However, the fact that significant changes were observed in a single growing season indicate that the effects of intercropping are realized rapidly and may accumulate over time to considerable changes in soil health and productivity.

As producers seek diverse agronomic practices to maintain soil health and productivity, intercropping may be a valuable strategy in some agricultural ecosystems. Continuing to monitor changes in soil health over a longer time frame will provide more robust information on trends that will help to guide decision-making processes.

References

Andrews, S. S., Karlen, D. L., & Cambardella, C. a. (2004). The Soil Management Assessment Framework: A quantitative soil quality evaluation method. *Soil Science Society of America Journal*, *68*(6), 1945–1962.

Aronoff, S. (1955). Translocation from Soybean Leaves, II. *Plant Physiology*, *30*(2), 184–185. https://doi.org/10.1104/pp.30.2.184

Avice, J. C., Ourry, A., Lemaire, G., & Boucaud, J. (1996). Nitrogen and carbon flows estimated by 15N and 13C pulse-chase labeling during regrowth of Alfalfa. *Plant Physiology*, *112*(1), 281–290. https://doi.org/10.1104/pp.112.1.281

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microbial Ecology*, *45*(4), 373–383. https://doi.org/10.1007/s00248-003-2002-y

Bedoussac, L., Journet, E. P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E. S., … Justes, E. (2015). Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agronomy for Sustainable Development*, *35*(3), 911–935. https://doi.org/10.1007/s13593-014-0277-7

Bennett, L. T., Mele, P. M., Annett, S., & Kasel, S. (2010). Examining links between soil management, soil health, and public benefits in agricultural landscapes: An Australian perspective. *Agriculture, Ecosystems and Environment*, *139*(1–2), 1–12. https://doi.org/10.1016/j.agee.2010.06.017

Biddulph, O., & Cory, R. (1960). Demonstration of Two Translocation Mechanisms in Studies of Bidirectional Movement. *Plant Physiology*, *35*(5), 689–695. https://doi.org/10.1104/pp.35.5.689

Bossio, D. A., & Scow, K. M. (1998). Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology*, Vol. 35, pp. 265–278. https://doi.org/10.1007/s002489900082

Bossio, D. A., Scow, K. M., Gunapala, N., & Graham, K. J. (1998). Determinants of Soil Microbial Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty Acid Profiles. *Microbial Ecology*, (36), 1–12.

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology*, *74*(3), 738–744. https://doi.org/10.1128/AEM.02188-07

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., … White, P. J. (2015). Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, Vol. 206, pp. 107–117. https://doi.org/10.1111/nph.13132

Brüggemann, N., Gessler, A., Kayler, Z., Keel, S. G., Badeck, F., Barthel, M., … Bahn, M. (2011). Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: A review. *Biogeosciences*, *8*(11), 3457–3489. https://doi.org/10.5194/bg-8-3457-2011

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., dos Santos, C. A., Alves, P. R. L., … Nogueira, M. A. (2013). Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, *70*(4), 274–289. https://doi.org/10.1590/S0103-90162013000400009

Carter, M. S., & Ambus, P. (2006). Biologically fixed N2 as a source for N2O production in a grass-clover mixture, measured by 15N2. *Nutrient Cycling in Agroecosystems*, *74*(1), 13–26. https://doi.org/10.1007/s10705-005-4111-0

Chalk, P. M., He, J. Z., Peoples, M. B., & Chen, D. (2017). 15N2 as a tracer of biological N2 fixation: A 75-year retrospective. *Soil Biology and Biochemistry*, *106*, 36–50. https://doi.org/10.1016/j.soilbio.2016.12.010

Chapagain, T., & Riseman, A. (2014). Barley-pea intercropping: Effects on land productivity, carbon and nitrogen transformations. *Field Crops Research*, *166*(September), 18–25. https://doi.org/10.1016/j.fcr.2014.06.014

Cherkasov, N., Ibhadon, A. O., & Fitzpatrick, P. (2015). A review of the existing and alternative methods for greener nitrogen fixation. *Chemical Engineering and Processing: Process Intensification*, Vol. 90, pp. 24–33. https://doi.org/10.1016/j.cep.2015.02.004

Clermont-Dauphin, C., Dissataporn, C., Suvannang, N., Pongwichian, P., Maeght, J. luc, Hammecker, C., & Jourdan, C. (2018). Intercrops improve the drought resistance of young rubber trees. *Agronomy for Sustainable Development*, Vol. 38. https://doi.org/10.1007/s13593-018-0537-z

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, *85*(3), 235–252. https://doi.org/10.1007/s10533-007-9132-0

Corre-Hellou, G., Fustec, J., & Crozat, Y. (2006). Interspecific competition for soil N and its interaction with N2 fixation, leaf expansion and crop growth in pea-barley intercrops. *Plant and Soil*, *282*(1–2), 195–208. https://doi.org/10.1007/s11104-005-5777-4

Coskun, D., Britto, D. T., & Kronzucker, H. J. (2016). Nutrient constraints on terrestrial carbon fixation: The role of nitrogen. *Journal of Plant Physiology*, *203*, 95–109. https://doi.org/10.1016/j.jplph.2016.05.016

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Cox, H. W., Kelly, R. M., & Strong, W. M. (2010). Pulse crops in rotation with cereals can be a profitable alternative to nitrogen fertiliser in central Queensland. *Crop and Pasture Science*, *61*(9), 752–762. https://doi.org/10.1071/CP09352

Culman, S., Freeman, M., & Snapp, S. (2012). Procedure for the Determination of Permanganate Oxidizable Carbon. *KBS POXC Protocol – Last Revised*, *1*, 1–5.

Dovrat, G., Bakhshian, H., Masci, T., & Sheffer, E. (2020). The nitrogen economic spectrum of legume stoichiometry and fixation strategy. *New Phytologist*, Vol. 227, pp. 365–375. https://doi.org/10.1111/nph.16543

Drake, D. C. (2011). Invasive legumes fix N2 at high rates in riparian areas of an N-saturated, agricultural catchment. *Journal of Ecology*, *99*(2), 515–523. https://doi.org/10.1111/j.1365-2745.2010.01787.x

Economic Impacts of Climate Change on Agriculture in Idaho. (2018). *The Impact of Climate Change on Our Life: The Questions of Sustainability*, 179–195. https://doi.org/10.1007/978-981-10-7748-7_10

Farquhar, G. D., Fasham, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., Kheshgi, H. S., … Wallace, D. W. R. (2001). The Carbon Cycle and Atmospheric Carbon Dioxide. In *IPCC, 2001: Climate Change 2001: The Scientific Basis.2*. https://doi.org/10.1029/eo067i015p00191

Frac, M., Hannula, S. E., Belka, M., & Jędryczka, M. (2018). Fungal biodiversity and their role in soil health. *Frontiers in Microbiology*, *9*(APR), 1–9. https://doi.org/10.3389/fmicb.2018.00707

Franzluebbers, A. J., Hons, F. M., & Zuberer, D. A. (1995). Tillage and Crop Effects on Seasonal Soil Carbon and Nitrogen Dynamics. *Soil Science Society of America Journal*, *59*(6), 1618–1624. https://doi.org/10.2136/sssaj1995.03615995005900060016x

Frostegard, A., & Baath, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, *22*(1–2), 59–65. https://doi.org/10.1007/bf00384433

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., & Richter, A. (2014). Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. *New Phytologist*, Vol. 201, pp. 916–927. https://doi.org/10.1111/nph.12569

Fustec, J., Lesuffleur, F., Mahieu, S., & Cliquet, J. B. (2009). Nitrogen rhizodeposition of legumes. *Sustainable Agriculture*, *2*, 869–881. https://doi.org/10.1007/978-94-007-0394-0_38

Galloway, J., Dentener, F., Capone, D. G., Boyer, E. W., Howarth, R. ., Seitzinger, S., … Vorosmarty, C. J. (2004). Nitrogen Cycles: past, present, and future. *Biogeochemistry*, *70*, 153–226.

Gan, Y., Hamel, C., O'Donovan, J. T., Cutforth, H., Zentner, R. P., Campbell, C. A., … Poppy, L. (2015). Diversifying crop rotations with pulses enhances system productivity. *Scientific Reports*, *5*, 1–14. https://doi.org/10.1038/srep14625

Geisen, S., Mitchell, E. A. D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., … Lara, E. (2018). Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Reviews*, *42*(3), 293–323. https://doi.org/10.1093/femsre/fuy006

Glaze-Corcoran, S., Hashemi, M., Sadeghpour, A., Jahanzad, E., Keshavarz Afshar, R., Liu, X., & Herbert, S. J. (2020). Understanding intercropping to improve agricultural resiliency and environmental sustainability. *Advances in Agronomy*, Vol. 162, pp. 199–256. https://doi.org/10.1016/bs.agron.2020.02.004

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362–2371. https://doi.org/10.1002/jsfa.6577

Hauggaard-Nielsen, H., Ambus, P., & Jensen, E. S. (2001). Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops - A field study employing 32p technique. *Plant and Soil*, *236*(1), 63–74. https://doi.org/10.1023/A:1011909414400

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., … Jensen, E. S. (2009). Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crops Research*, *113*(1), 64–71. https://doi.org/10.1016/j.fcr.2009.04.009

Hauggaard-Nielsen, H., & Jensen, E. S. (2005). Facilitative root interactions in intercrops. *Plant and Soil*, *274*(1–2), 237–250. https://doi.org/10.1007/s11104-004-1305-1

Hauggaard-Nielsen, Henrik, Ambus, P., & Jensen, E. S. (2001). Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research*, *70*, 101–109.

Høgh-Jensen, H. (2006). The nitrogen transfer between plants: An important but difficult flux to quantify. *Plant and Soil*, *282*(1–2), 1–5. https://doi.org/10.1007/s11104-005-2613-9

Houghton, R. A. (2003). The Contemporary Carbon Cycle. In *Treatise on Geochemistry* (pp. 473–513).

Huang, X., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., & Vivanco, J. M. (2014). *Rhizosphere interactions: root exudates, microbes, and microbial communities 1*. *275*(February), 267–275.

IFA – Internationl Fertilizer Association. (2021). Public Summary Medium-Term Fertilizer Outlook 2021 – 2025. *Public Summary Medium-Term Fertilizer Outlook 2021 – 2025. IFA, 2021.*, *1*(August), 1–9. Retrieved from https://www.ifastat.org/

Jenkinson, D. S. (2001). The impact of humans on the nitrogen cycle, with focus on temperate arable agriculture. *Plant and Soil*, *228*(1), 3–15. https://doi.org/10.1023/A:1004870606003

Jensen, E. S. (1996). Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil*, *182*(1), 25–38.

Jiang-shan, Z., Jian-fen, G., Guang-shui, C., & Wei, Q. (2005). Soil microbial biomass and its controls. *Journal of Forestry Research*, *16*(4), 327–330. https://doi.org/10.1007/bf02858201

Jiao, S., Wang, J., Wei, G., Chen, W., & Lu, Y. (2019). Dominant role of abundant rather than rare bacterial taxa in maintaining agro-soil microbiomes under environmental disturbances. *Chemosphere*, *235*, 248–259. https://doi.org/10.1016/j.chemosphere.2019.06.174

Johansen, A., & Jensen, E. S. (1996). Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology and Biochemistry*, *28*(1), 73–81. https://doi.org/10.1016/0038-0717(95)00117-4

Johnson, P. T. J., Townsend, A. R., Cleveland, C. C., Glibert, P. M., Howarth, R. W., Mckenzie, V. J., … Ward, M. H. (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. *Ecological Applications*, Vol. 20, pp. 16–29. https://doi.org/10.1890/08-0633.1

Jones, H., Martin, R. V., & Porter, H. K. (1959). Translocation of ^{14}Carbon in Tobacco following Assimilation of ^{14}Carbon Dioxide by a Single Leaf. *Annals of Botany*, *23*(92), 493–508.

Kayler, Z., Keitel, C., Jansen, K., & Gessler, A. (2017). Experimental evidence of two mechanisms coupling leaf-level C assimilation to rhizosphere CO2 release. *Environmental and Experimental Botany*, *135*, 21–26. https://doi.org/10.1016/j.envexpbot.2016.12.002

Keeling, C. D., Piper, S. C., Bacastow, R. B., Wahlen, M., Whorf, T. P., Heimann, M., & Meijer, H. A. (2005). Terrestrial Biosphere and Oceans from 1978 to 2000 : Observations and Carbon Cycle Implications. *A History of Atmospheric CO2 and Its Effects on Plants, Animals, and Ecosystems*, 83–113.

Koong, D., Benz, S., Johnson, B., Losh, D., Stebbins, E., & Reichert, J. (2020). 2020 Idaho Annual Statistical Bulletin. *USDA National Agricultural Statistics Service*.

Lal, R. (2014). Societal value of soil carbon. *Journal of Soil and Water Conservation*, *69*(6), 186A-192A. https://doi.org/10.2489/jswc.69.6.186A

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability (Switzerland)*, Vol. 7, pp. 5875–5895. https://doi.org/10.3390/su7055875

LeBauer, D; Treseder, K. (2008). Nitrogen Limitation of Net Primary Productivity. *Ecology*, *89*(2), 371–379.

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, *70*(1), 12A-18A. https://doi.org/10.2489/jswc.70.1.12A

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth and Environment*, Vol. 1, pp. 544–553. https://doi.org/10.1038/s43017-020-0080-8

Lesuffleur, F., Salon, C., Jeudy, C., & Cliquet, J. B. (2013). Use of a 15N2 labelling technique to estimate exudation by white clover and transfer to companion ryegrass of symbiotically fixed N. *Plant and Soil*, *369*(1–2), 187–197. https://doi.org/10.1007/s11104-012-1562-3

Li, C., Cano, A., Acosta-Martinez, V., Veum, K. S., & Moore-Kucera, J. (2020). A comparison between fatty acid methyl ester profiling methods (PLFA and EL-FAME) as soil health indicators. *Soil Science Society of America Journal*, *84*(4), 1153–1169. https://doi.org/10.1002/saj2.20118

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. A., & Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia*, *147*(2), 280–290. https://doi.org/10.1007/s00442-005-0256-4

Lian, T., Mu, Y., Jin, J., Ma, Q., Cheng, Y., Cai, Z., & Nian, H. (2019). Impact of intercropping on the coupling between soil microbial community structure, activity, and nutrient-use efficiencies. *PeerJ*, *2019*(2), 1–18. https://doi.org/10.7717/peerj.6412

Lithourgidis, A. S., Dordas, C. A., Damalas, C. A., & Vlachostergios, D. N. (2011). Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop Science*, *5*(4), 396–410.

Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth System Science Data*, *9*(1), 181–192. https://doi.org/10.5194/essd-9-181-2017

Luo, X., Keenan, T. F., Chen, J. M., Croft, H., Colin Prentice, I., Smith, N. G., … Zhang, Y. (2021). Global variation in the fraction of leaf nitrogen allocated to photosynthesis. *Nature Communications*, *12*(1), 1–10. https://doi.org/10.1038/s41467-021-25163-9

Malik, A. A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P. G. M., … Gleixner, G. (2016). Soil fungal: Bacterial ratios are linked to altered carbon cycling. *Frontiers in Microbiology*, *7*(AUG), 1–11. https://doi.org/10.3389/fmicb.2016.01247

Mann, C., Lynch, D., Fillmore, S., & Mills, A. (2019). Relationships between field management, soil health, and microbial community composition. *Applied Soil Ecology*, *144*(December 2018), 12–21. https://doi.org/10.1016/j.apsoil.2019.06.012

Manninen, N., Soinne, H., Lemola, R., Hoikkala, L., & Turtola, E. (2018). Effects of agricultural land use on dissolved organic carbon and nitrogen in surface runoff and subsurface drainage. *Science of the Total Environment*, *618*, 1519–1528. https://doi.org/10.1016/j.scitotenv.2017.09.319

McConnell, M., Liefert, O., Williams, A., & Boline, A. (2022). Feed Outlook: January 2022. *UDSA*

Economic Research Service Situation and Outlook Report, *FDS-22a*(January).

Menge, D. N. L., Levin, S. A., & Hedin, L. O. (2009). Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. *American Naturalist*, *174*(4), 465–477. https://doi.org/10.1086/605377

Menge, D. N. L., Wolf, A. A., & Funk, J. L. (2015). Diversity of nitrogen fixation strategies in Mediterranean legumes. *Nature Plants*, *1*(6), 1–5. https://doi.org/10.1038/nplants.2015.64

Millar, N., Urrea, A., Kahmark, K., Shcherbak, I., Robertson, G. P., & Ortiz-Monasterio, I. (2018). Nitrous oxide (N2O) flux responds exponentially to nitrogen fertilizer in irrigated wheat in the Yaqui Valley, Mexico. *Agriculture, Ecosystems and Environment*, *261*(April), 125–132. https://doi.org/10.1016/j.agee.2018.04.003

Miller, P. R., McConkey, B. G., Clayton, G. W., Brandt, S. A., Staricka, J. A., Johnston, A. M., … Neill, K. E. (2002). Pulse crop adaptation in the northern Great Plains. *Agronomy Journal*, *94*(2), 261–272. https://doi.org/10.2134/agronj2002.0261

Minchin, F. R., & Pate, J. S. (1973). The carbon balance of a legume and the functional economy of its root nodules. *Journal of Experimental Botany*, *24*(2), 259–271. https://doi.org/10.1093/jxb/24.2.259

Moebius-Clune, B. N., Moebius-Clune, D. J., Gugino, B. K., Idowu, O. J., Schindelbeck, R. R., Ristow, A. J., … Abawi, G. S. (2016). Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.1. In *Cornell University, Geneva, NY*.

Moore-Kucera, J., & Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Microbial Ecology*, *55*(3), 500–511. https://doi.org/10.1007/s00248-007-9295-1

Moyer-Henry, K. A., Burton, J. W., Israel, D. W., & Rufty, T. W. (2006). Nitrogen transfer between plants: A 15N natural abundance study with crop and weed species. *Plant and Soil*, *282*(1–2), 7–20. https://doi.org/10.1007/s11104-005-3081-y

National Agricultural Statistics Service, U. (2020). *Small Grains 2020 Summary*. (September).

Naylor, D., & Coleman-Derr, D. (2018). Drought stress and root-associated bacterial communities. *Frontiers in Plant Science*, *8*(January), 1–16. https://doi.org/10.3389/fpls.2017.02223

Norris, C. E., Bean, G. Mac, Cappellazzi, S. B., Cope, M., Greub, K. L. H., Liptzin, D., … Honeycutt, C. W. (2020). Introducing the North American project to evaluate soil health measurements. *Agronomy Journal*, Vol. 112, pp. 3195–3215. https://doi.org/10.1002/agj2.20234

Outlaw, J. L., Bryant, H. L., Raulston, J. M., Knapek, G. M., Herbst, B. K., & Fischer, B. L. (2022). *Economic Impact of Higher Fertilizer Prices on AFPC's Representative Crop Farms*.

Paynel, F., Murray, P. J., & Bernard Cliquet, J. (2001). Root exudates: A pathway for short-term N transfer from clover and ryegrass. *Plant and Soil*, *229*(2), 235–243. https://doi.org/10.1023/A:1004877214831

Peoples, M. B., Giller, K. E., Jensen, E. S., & Herridge, D. F. (2021). Quantifying country-to-global scale nitrogen fixation for grain legumes: I. Reliance on nitrogen fixation of soybean, groundnut and pulses. *Plant and Soil*, *469*(1–2), 1–14. https://doi.org/10.1007/s11104-021-05167-6

Pikaar, I., Matassa, S., Rabaey, K., Bodirsky, B. L., Popp, A., Herrero, M., & Verstraete, W. (2017). Microbes and the Next Nitrogen Revolution. *Environmental Science and Technology*, *51*(13),

7297–7303. https://doi.org/10.1021/acs.est.7b00916

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., & Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, *350*(1–2), 71–84. https://doi.org/10.1007/s11104-011-0882-z

Quideau, S. A., McIntosh, A. C. S., Norris, C. E., Lloret, E., Swallow, M. J. B., & Hannam, K. (2016). Extraction and analysis of microbial Phospholipid fatty acids in soils. *Journal of Visualized Experiments*, *2016*(114), 1–9. https://doi.org/10.3791/54360

Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*(1–2), 341–356. https://doi.org/10.1007/s11104-004-0907-y

Renwick, L. L. R., Kimaro, A. A., Hafner, J. M., Rosenstock, T. S., & Gaudin, A. C. M. (2020). Maize-Pigeonpea Intercropping Outperforms Monocultures Under Drought. *Frontiers in Sustainable Food Systems*, *4*(December). https://doi.org/10.3389/fsufs.2020.562663

Rodriguez, C., Carlsson, G., Englund, J. E., Flöhr, A., Pelzer, E., Jeuffroy, M. H., … Jensen, E. S. (2020). Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. *European Journal of Agronomy*, *118*(July 2019). https://doi.org/10.1016/j.eja.2020.126077

Rousk, J., Brookes, P. C., & Bååth, E. (2010). The microbial PLFA composition as affected by pH in an arable soil. *Soil Biology and Biochemistry*, *42*(3), 516–520. https://doi.org/10.1016/j.soilbio.2009.11.026

Savci, S. (2012). Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia*, *1*(January), 287–292. https://doi.org/10.1016/j.apcbee.2012.03.047

Schaefer, J., Kier, L. D., & Stejskal, E. O. (1980). Characterization of Photorespiration in Intact Leaves Using 13 Carbon Dioxide Labeling . *Plant Physiology*, *65*(2), 254–259. https://doi.org/10.1104/pp.65.2.254

Schmidt, E. L., Bakole, R. O., & Bohlool, B. B. (1968). Fluorescent-antibody approach to study of rhizobia in soil. *Journal of Bacteriology*, *95*(6), 1987–1992. https://doi.org/10.1128/jb.95.6.1987-1992.1968

Schulze, J. (2004). How are nitrogen fixation rates regulated in legumes? *Journal of Plant Nutrition and Soil Science*, *167*(2), 125–137. https://doi.org/10.1002/jpln.200320358

Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N 2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(25), 9199–9204. https://doi.org/10.1073/pnas.1322434111

Shestak, C. J., & Busse, M. D. (2005). Compaction Alters Physical but Not Biological Indices of Soil Health. *Soil Science Society of America Journal*, Vol. 69, p. 236. https://doi.org/10.2136/sssaj2005.0236

Smil, V. (1999). Nitrogen in crop production : An account of global flows adds. *Global Biogeochemical Cycles*, *13*(2), 647–662.

Stehfest, E., & Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems*, *74*, 207–228.

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., … Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, Vol. 164, pp. 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Strickland, M. S., & Rousk, J. (2010). Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Tavi, N. M., Martikainen, P. J., Lokko, K., Kontro, M., Wild, B., Richter, A., & Biasi, C. (2013). Linking microbial community structure and allocation of plant-derived carbon in an organic agricultural soil using 13CO2 pulse-chase labelling combined with 13C-PLFA profiling. *Soil Biology and Biochemistry*, *58*, 207–215. https://doi.org/10.1016/j.soilbio.2012.11.013

Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A., & Raizada, M. N. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*, *36*(4). https://doi.org/10.1007/s13593-016-0396-4

Tian, D., & Niu, S. (2015). A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters*, *10*(2). https://doi.org/10.1088/1748-9326/10/2/024019

Tomè, E., Tagliavini, M., & Scandellari, F. (2015). Recently fixed carbon allocation in strawberry plants and concurrent inorganic nitrogen uptake through arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, *179*, 83–89. https://doi.org/10.1016/j.jplph.2015.02.008

Townsend, A. R., & Howarth, R. W. (2010). Fixing the Global Nitrogen Problem. *Scientific American*, *302*(2), 64–71.

Turgeon, R. (1989). The sink-source transition in leaves. *Annual Review of Plant Physiology*, *40*(1), 119–138. https://doi.org/10.1146/annurev.biochem.64.1.721

von Rein, I., Gessler, A., Premke, K., Keitel, C., Ulrich, A., & Kayler, Z. E. (2016). Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. *Global Change Biology*, *22*(8), 2861–2874. https://doi.org/10.1111/gcb.13270

Wade, J., Maltais-Landry, G., Lucas, D. E., Bongiorno, G., Bowles, T. M., Calderón, F. J., … Margenot, A. J. (2020). Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils. *Geoderma*, *366*(December 2019), 114235. https://doi.org/10.1016/j.geoderma.2020.114235

Walker, S., & Ogindo, H. O. (2003). The water budget of rainfed maize and bean intercrop. *Physics and Chemistry of the Earth*, *28*(20–27), 919–926. https://doi.org/10.1016/j.pce.2003.08.018

Wan, X., Huang, Z., He, Z., Yu, Z., Wang, M., Davis, M. R., & Yang, Y. (2015). Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. *Plant and Soil*, *387*(1–2), 103–116. https://doi.org/10.1007/s11104-014-2277-4

Wang, S. L., Nehring, R., & Mosheim, R. (2018). Agricultural Productivity Growth in the United States: 1948-2015. *USDA, Economic Research Service, Amber Waves Magazine*.

Wardle, D. A. (1998). Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. *Soil Biology and Biochemistry*, *30*(13), 1627–1637. https://doi.org/10.1016/S0038-0717(97)00201-0

Weiner, J., Campbell, L. G., Pino, J., & Echarte, L. (2009). The allometry of reproduction within plant populations. *Journal of Ecology*, Vol. 97, pp. 1220–1233. https://doi.org/10.1111/j.1365-2745.2009.01559.x

Xiao, Y., Li, L., & Zhang, F. (2004). Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15N techniques. *Plant and Soil*, *262*(1–2), 45–54. https://doi.org/10.1023/B:PLSO.0000037019.34719.0d

Yang, T., Siddique, K. H. M., & Liu, K. (2020). Cropping systems in agriculture and their impact on soil health-A review. *Global Ecology and Conservation*, *23*, e01118. https://doi.org/10.1016/j.gecco.2020.e01118

Yuste, J. C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., … Sardans, J. (2011). Drought-resistant fungi control soil organic matter decomposition and its response to temperature. *Global Change Biology*, *17*(3), 1475–1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. *Biology and Fertility of Soils*, *29*(2), 111–129. https://doi.org/10.1007/s003740050533

Zhao, H., Li, X., Zhang, Z., Zhao, Y., Chen, P., & Zhu, Y. (2018). Drivers and assemblies of soil eukaryotic microbes among different soil habitat types in a semi-arid mountain in China. *PeerJ*, Vol. 2018. https://doi.org/10.7717/peerj.6042

Zosso, C. U., & Wiesenberg, G. L. B. (2021). Methylation procedures affect PLFA results more than selected extraction parameters. *Journal of Microbiological Methods*, *182*, 106164. https://doi.org/10.1016/j.mimet.2021.106164

Supplemental Information

Supplemental Table 2.1: Identified PLFAs and their microbial group assignments (Figure 2.6).

Microbial Group	PLFAs
General FAME	16:0 aldehyde, 16:1ω7c alcohol, 16:0 N alcohol
AM Fungi	16:1ω5c
Gram-Negative Bacteria	12:1ω8c, 14:1ω7c, 15:1ω7c, 16:1ω9c, 17:1ω8c, 16:1ω6c, 17:1ω7c, 17:0 cyclo ω7c, 17:1ω4c, 17:1ω3c, 18:1ω7c, 18:1ω5c, 19:1ω8c, 19:0 cyclo ω7c, 17:0 iso 3OH
Eukaryotes	15:4ω3c, 18:3ω6c, 19:4ω6c, 19:3ω6c, 19:3ω3c, 18:4ω3c, 20:4ω6c, 20:5ω3c, 20:3ω6c, 20:2ω6c, 22:2ω6c, 22:4ω6c
Fungi	18:2ω6c, 18:1ω9c
Gram-Positive Bacteria	13:0 iso, 14:0 iso, 15:1 iso ω9c, 15:1 iso ω6c, 15:1 anteiso ω9c, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0 anteiso, 17:1 iso ω9c, 17:0 anteiso, 18:0 iso, 17:1 anteiso ω9c, 17:1 iso ω10c, 19:0 iso, 19:0 anteiso
Anaerobes	15:0 DMA, 16:2 DMA, 16:1ω7c DMA, 16:0 DMA, 18:1ω9c DMA
Actinomycetes	16:0 10-methyl, 17:1ω7c 10-methyl, 17:0 10-methyl, 18:1ω7c 10-methyl, 18:0 10-methyl
Protozoa	20:4ω6c, 20:3ω6c

Supplemental Table 2.2: Soil carbon change (amount and percent)

Crop	Irrigation	Depth	Absolute Change (mg/kg soil)	Percent Change
BO	Deficit Irrigation	0-15cm	2.06	5.71
BO	Full Irrigation	0-15cm	52.92	56.39
BO	Deficit Irrigation	15-30cm	-3.74	-11.28
BO	Full Irrigation	15-30cm	13.83	18.04
BO	Deficit Irrigation	30-60cm	7.33	-38.88
BO	Full Irrigation	30-60cm	13.04	110.92
BO	Deficit Irrigation	60-90cm	26.17	354.02
BO	Full Irrigation	60-90cm	27.05	331.01
LB	Deficit Irrigation	0-15cm	0.71	1.99
LB	Full Irrigation	0-15cm	-35.54	-43.70
LB	Deficit Irrigation	15-30cm	-23.35	-29.41
LB	Full Irrigation	15-30cm	-11.01	-14.95
LB	Deficit Irrigation	30-60cm	3.77	33.13
LB	Full Irrigation	30-60cm	17.56	87.87
LB	Deficit Irrigation	60-90cm	5.05	1.17
LB	Full Irrigation	60-90cm	21.39	321.69
LO	Deficit Irrigation	0-15cm	13.21	16.96
LO	Full Irrigation	0-15cm	26.25	33.99

LO	Deficit Irrigation	15-30cm	-4.50	-3.98
LO	Full Irrigation	15-30cm	21.45	27.77
LO	Deficit Irrigation	30-60cm	11.62	35.12
LO	Full Irrigation	30-60cm	28.52	130.19
LO	Deficit Irrigation	60-90cm	28.29	127.58
LO	Full Irrigation	60-90cm	19.19	210.03
PB	Deficit Irrigation	0-15cm	-0.37	-0.17
PB	Full Irrigation	0-15cm	20.29	31.28
PB	Deficit Irrigation	15-30cm	-43.73	-13.61
PB	Full Irrigation	15-30cm	6.73	12.88
PB	Deficit Irrigation	30-60cm	23.13	152.93
PB	Full Irrigation	30-60cm	23.85	105.81
PB	Deficit Irrigation	60-90cm	21.57	350.71
PB	Full Irrigation	60-90cm	8.69	130.80
PO	Deficit Irrigation	0-15cm	5.88	8.73
PO	Full Irrigation	0-15cm	14.56	18.89
PO	Deficit Irrigation	15-30cm	0.96	3.39
PO	Full Irrigation	15-30cm	13.46	16.91
PO	Deficit Irrigation	30-60cm	37.48	156.23
PO	Full Irrigation	30-60cm	38.19	84.27
PO	Deficit Irrigation	60-90cm	8.12	187.91
PO	Full Irrigation	60-90cm	27.93	532.41

Supplemental Table 2.3: Soil inorganic nitrogen change (amount and percent)

Crop	Irrigation	Depth	Absolute Change (mg/kg soil)	Percent Change
BO	Deficit Irrigation	0-15cm	-7.46	-68.11
BO	Full Irrigation	0-15cm	-4.88	-33.34
BO	Deficit Irrigation	15-30cm	-9.28	-48.24
BO	Full Irrigation	15-30cm	-6.86	-47.32
BO	Deficit Irrigation	30-60cm	-15.68	-67.54
BO	Full Irrigation	30-60cm	-22.81	-44.49
BO	Deficit Irrigation	60-90cm	-6.10	-40.84
BO	Full Irrigation	60-90cm	-6.52	-46.63
LB	Deficit Irrigation	0-15cm	-5.64	-47.60
LB	Full Irrigation	0-15cm	-7.24	-63.61
LB	Deficit Irrigation	15-30cm	-14.41	-72.17
LB	Full Irrigation	15-30cm	-7.57	-54.07
LB	Deficit Irrigation	30-60cm	-11.79	-61.49
LB	Full Irrigation	30-60cm	-13.64	-37.76
LB	Deficit Irrigation	60-90cm	-2.53	-37.16
LB	Full Irrigation	60-90cm	-7.47	9.84
LO	Deficit Irrigation	0-15cm	-3.38	-37.53
LO	Full Irrigation	0-15cm	-7.51	-41.50
LO	Deficit Irrigation	15-30cm	-4.17	-37.38
LO	Full Irrigation	15-30cm	-16.16	-43.79
LO	Deficit Irrigation	30-60cm	-4.80	-28.00
LO	Full Irrigation	30-60cm	-14.45	-67.66
LO	Deficit Irrigation	60-90cm	-0.38	6.88
LO	Full Irrigation	60-90cm	1.60	6.94
PB	Deficit Irrigation	0-15cm	-7.76	-71.03
PB	Full Irrigation	0-15cm	-7.43	-58.29
PB	Deficit Irrigation	15-30cm	-12.05	-75.09
PB	Full Irrigation	15-30cm	3.76	12.63
PB	Deficit Irrigation	30-60cm	-15.00	-68.54
PB	Full Irrigation	30-60cm	-18.29	-72.82
PB	Deficit Irrigation	60-90cm	-9.85	-59.65
PB	Full Irrigation	60-90cm	-3.17	-30.13
PO	Deficit Irrigation	0-15cm	-6.87	-54.10
PO	Full Irrigation	0-15cm	-9.15	-62.05
PO	Deficit Irrigation	15-30cm	-5.28	-39.47
PO	Full Irrigation	15-30cm	-7.32	-49.17
PO	Deficit Irrigation	30-60cm	-10.84	-48.85
PO	Full Irrigation	30-60cm	-5.70	-40.35
PO	Deficit Irrigation	60-90cm	-8.81	-40.08
PO	Full Irrigation	60-90cm	-4.69	23.42

Supplemental Table 2.4: Cumulative soil moisture to 60cm for each crop and irrigation strategy across the entire growing season. BO = barley only, LB = lentil/barley, LO = lentil only, PB = pea/barley, PO = pea only. Intercropping (LB, PB) resulted in lower soil moisture than monocropped barley, suggesting more of the available water in the soil profile was used with intercropping.

Treatment	Cumulative Moisture (%)
BO Full Irrigation	1927.2
LB Full Irrigation	1728.8
LO Full Irrigation	1654.1
PB Full Irrigation	1587.3
BO Deficit Irrigation	966.9
PO Deficit Irrigation	1263.5

Supplemental Table 2.5: Average biomass by crop treatment and component crop in g/m^2.

Crop Treatment	Irrigation	Barley Biomass (g/m^2)	Std. error	Pulse Biomass (g/m^2)	Std. error	Total Biomass (g/m^2)	Std. error
Barley Monocrop	Full	2387.5	206.53	0	*NA*	2387.5	206.53
Lentil Monocrop	Full	0	*NA*	1504.2	126.55	1504.2	126.55
Pea Monocrop	Full	0	*NA*	1622.2	70.09	1622.2	70.09
Barley/Lentil	Full	1532.4	145.16	235.2	89.64	1777.6	63.84
Barley/Pea	Full	1660.2	292.06	273.1	96.76	1933.3	227.98
Barley Monocrop	Deficit	1625.0	109.82	0	*NA*	1625.0	109.82
Lentil Monocrop	Deficit	0	*NA*	1287.5	51.44	1287.5	51.44
Pea Monocrop	Deficit	0	*NA*	1481.9	286.29	1481.9	286.29
Barley/Lentil	Deficit	1343.1	56.82	154.9	34.85	1498.0	45.34
Barley/Pea	Deficit	1414.8	267.09	171.3	6.48	1586.1	263.25

Supplemental Table 2.6: Explained variance of variables by PLS components.

	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16	t17
Crop	0.09	0.13	0.17	0.24	0.63	0.77	0.81	0.94	0.96	0.96	0.98	0.98	0.98	0.99	0.99	1.00	1.00
Water	0.02	0.14	0.14	0.19	0.22	0.22	0.23	0.33	0.34	0.43	0.53	0.53	0.62	0.99	0.99	1.00	1.00
Yield	0.00	0.21	0.25	0.29	0.30	0.58	0.61	0.84	0.93	0.93	0.94	0.94	0.95	0.99	0.99	1.00	1.00
C1	0.05	0.24	0.25	0.55	0.75	0.80	0.83	0.85	0.97	0.98	0.98	0.98	0.98	1.00	1.00	1.00	1.00
C2	0.39	0.43	0.43	0.73	0.79	0.80	0.80	0.86	0.87	0.88	0.90	0.91	0.91	0.94	0.94	1.00	1.00
C3	0.27	0.39	0.39	0.40	0.42	0.70	0.70	0.71	0.82	0.87	0.87	0.87	0.90	0.96	0.97	1.00	1.00
C4	0.01	0.02	0.24	0.24	0.42	0.51	0.65	0.72	0.76	0.84	0.98	0.98	0.99	1.00	1.00	1.00	1.00
Root Mass	0.45	0.56	0.63	0.70	0.71	0.71	0.73	0.85	0.87	0.91	0.91	0.91	0.95	0.95	0.98	1.00	1.00
pH	0.01	0.02	0.33	0.58	0.58	0.59	0.72	0.75	0.78	0.85	0.89	0.89	0.90	0.97	0.98	1.00	1.00
C:N 1	0.16	0.41	0.42	0.44	0.44	0.64	0.90	0.94	0.95	0.96	0.98	0.98	0.98	0.99	0.99	1.00	1.00
C:N 2	0.51	0.74	0.80	0.83	0.85	0.85	0.87	0.87	0.87	0.90	0.90	0.90	0.98	0.98	1.00	1.00	1.00
C:N 3	0.06	0.42	0.49	0.51	0.52	0.53	0.53	0.58	0.61	0.63	0.83	0.94	0.98	0.98	0.99	1.00	1.00
C:N 4	0.11	0.31	0.43	0.47	0.48	0.55	0.55	0.69	0.71	0.95	0.96	0.97	0.99	1.00	1.00	1.00	1.00
N1	0.31	0.32	0.37	0.38	0.39	0.73	0.82	0.95	0.96	0.97	0.97	0.98	0.98	0.98	0.99	1.00	1.00
N2	0.08	0.18	0.30	0.37	0.39	0.52	0.53	0.59	0.61	0.76	0.90	0.90	0.90	0.98	0.99	1.00	1.00
N3	0.01	0.25	0.52	0.62	0.63	0.71	0.74	0.80	0.81	0.96	0.97	0.99	1.00	1.00	1.00	1.00	1.00
N4	0.02	0.39	0.54	0.66	0.70	0.75	0.75	0.75	0.76	0.96	0.96	0.96	0.99	1.00	1.00	1.00	1.00
Biomass	0.34	0.45	0.51	0.55	0.58	0.59	0.59	0.59	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60

Chapter 3: Pulse-Chase Isotope Labeling to Trace Carbon and Nitrogen Flow Through Crop and Microbial Pools

Introduction

The spatial and temporal proximity of intercropped agricultural systems allows for more direct interactions between one or more plant species than in typical rotation systems. Soil nutrient status measurements taken prior to planting and after harvest illustrate net changes across the growing season. However, these bookend values do not reflect the actual flux dynamics that culminate in the observed changes across a growing season. Nutrient cycles driven largely by crops and their symbionts are dynamic in magnitude and direction and may reflect the priorities of various organisms at different times.

Using stable isotope labeling in pulse/barley intercropping systems allows us to understand how the addition of pulses influences the availability and allocation of nutrients within a narrow temporal scope. This enables the visualization of interactions between the atmosphere, crops, and the soil microbial community within the same growing season. Tracking the cycling of carbon (C) and nitrogen (N), critical elements for both plants and microbes provides an understanding of how pulse crops interact with plants of different species and soil microbes.

Linked C and N cycles

Broadly, C cycling is synonymous with the cycling of energy (Houghton, 2003). Understanding the C cycle in the context of an agroecosystem allows us to understand where plants are transferring organic carbon (energy), and where it is being taken up. Both plants and microbes utilize organic C compounds derived from photosynthesis to fuel biological processes. Plants allocate photosynthates among various tissues including roots, leaves, and shoots; soil microbes obtain organic C largely from root exudates and the decomposition of plant necromass in the soil (Gougoulias, Clark, & Shaw, 2014). The photosynthetic fixation of atmospheric carbon dioxide (CO_2) to organic forms is the source of energy for all life in the biosphere. This interaction between the atmosphere and terrestrial plants is an enormous flux, estimated at 120PgC/year, approximately a sixth of the entire atmospheric pool of C (Farquhar et al., 2001).

Despite the enormous pool of atmospheric C, its flow into the biosphere is largely limited by the availability of N, which is a limiting nutrient for plant growth nearly universally across terrestrial ecosystems (Coskun, Britto, & Kronzucker, 2016; LeBauer, D; Treseder, 2008). Plants require N for

photosynthesis, where it is primarily invested in the enzyme RuBisCO (Luo et al., 2021). As such, the cycles of C and N are inseparably coupled and provide feedback to one another.

The coupling of the C and N cycles is illustrated well by the example of biological nitrogen fixation (BNF) by *Rhizobium* bacteria, which form a symbiotic relationship with pulse crop roots. This relationship allows the conversion of inert N_2 gas, the largest component of the atmosphere, into a plant-available form (NH_4^+). The amount of N provided to pulse crops from fixation varies widely based on environmental conditions, but has been estimated to average 60% globally (Peoples, Giller, Jensen, & Herridge, 2021). Pulse crops may rely even more heavily on BNF when grown with cereal crops such as barley, which have been demonstrated to be more competitive for soil inorganic N than pulses (Corre-Hellou et al., 2006).

However, nitrogen fixation is an energetically costly process compared to the uptake of soil N, so the rate at which it can occur is largely controlled by the carbon (energy) supply to *Rhizobium* (Schulze, 2004). Pulse crops will allocate a substantial amount of their fixed C budget to providing these bacteria with C-rich photosynthates, possibly upwards of 17% (Minchin & Pate, 1973). In return, the plant receives N in an available form without relying on soil N pools, which are often limited.

In this symbiotic interaction, C and N flows act as a type of currency, and the availability and transfer of each nutrient largely dictates the cycling of the other. While separate elements, the C and N cycles are inextricably intertwined, and cannot be considered in full separately from one another.

Pulse crops

Pulse crops are often included in rotations because they increase plant-available soil N in subsequent growing seasons. Fixed N accumulates in plant tissues over the growing season, and root rhizodeposition combined with the decomposition of residue left on the field release N to the soil, where it is available for the next crop. This phenomenon is well-established, and as such rotating pulses with cereals is commonly practiced by producers worldwide, including in our study area in southern Idaho (Cox, Kelly, & Strong, 2010; Gan et al., 2015; Miller et al., 2002).

What remains less well-known is the impact on N-availability of growing pulse crops concurrently with cereals, rather than in rotation. The two crops may utilize distinct N pools, with pulses relying to some degree on BNF, thereby reducing competition with barley for soil N and resulting in more efficient use of N (Fustec et al., 2009). Additionally, rather than waiting for the growth, harvest, and decomposition of pulses to release N into the soil, it is possible that these crops can actively transfer

fixed N during the growing season to their companion crop (Carter & Ambus, 2006; Lesuffleur et al., 2013; Moyer-Henry, Burton, Israel, & Rufty, 2006; Paynel, Murray, & Bernard Cliquet, 2001).

The mechanism of N transfer to barley can be indirect, as the uptake of N compounds exuded into the rhizosphere by the pulse crop, or direct via arbuscular-mycorrhizal fungi which connect the roots of both crops (Paynel et al., 2001). While decomposition of sloughed root cells from the pulse crop may provide additional N during the growing season, it is unlikely this is a significant avenue of transfer over the period of days to weeks. Regardless of the mechanism, transfer of this nature is an example of facilitation, wherein "plants ameliorate the environment of their neighbors, and increase their growth and survival" that could benefit producers (H. Hauggaard-Nielsen & Jensen, 2005).

N management

Given its status as a limiting nutrient for plant growth, the importance of N management in agroecosystems can hardly be overstated. The dramatic increase in yields associated with the availability of inorganic N fertilizer from the Haber-Bosch process has supported the growth of the global population from approximately 1.5 billion to nearly 8 billion people in 2022. It is estimated that Haber-Bosch-derived N is the source of nearly three times as much N in the plant-based food chain as biologically-fixed N (Pikaar et al., 2017).

Undoubtedly, fertilizer was a key part of the Green Revolution and has benefitted the food security of the vast majority of the world. However, overuse and misapplication of fertilizer represents a large cost both to producers as well as the environment. Fertilizers can constitute upwards of 30% of total operating costs for producers based on the crop, and dramatic price increases in recent years have increased this burden (McConnell et al., 2022). Exacerbating the issue is the fact that up to half of fertilizer N applied to agricultural fields may not be taken up by crops (Smil, 1999). The consequences of this excess N entering the environment are wide-ranging and include soil acidification, waterway eutrophication, and increased greenhouse gas (N_2O) emissions (Galloway et al., 2004; Millar et al., 2018; Tian & Niu, 2015).

These factors make it clear why offsetting some fertilizer needs through methods such as pulse rotations and intercropping may appeal to producers as a way to increase soil N with less potential for financial and environmental risk. Tracing the flow of nutrients through a pulse-barley intercropping system using stable isotopes will allow an evaluation of the impacts on soil nutrient status to more fully understand the potential of intercropping.

Stable isotope labeling

Introducing C and N that are enriched in the heavy stable isotopes (^{13}C and ^{15}N) to an ecosystem creates a signature that is isotopically distinct from natural atmospheric pools. As the plant fixes these nutrients, this "label" can then be traced as it is transformed and transported through different pools within the ecosystem. Sampling pulse and barley crop tissues as well as microbial communities during the chase period allows us a visualization of the connectedness of these pools and their interactions over short time periods within a single growing season.

Hypotheses

Plant growth cannot occur without the uptake of CO_2 by photosynthesis; we hypothesize that labeled ^{13}C will enter the pulse crop leaf tissue and the resulting labeled photosynthates will be transferred to the roots of the pulse crop. Some amount of this labeled C will exit the plant roots as exudates, and be taken up by soil microbial communities, where it can be detected. We expect that C allocation to the microbial communities will vary based on pulse species (pea or lentil); the magnitude of microbial enrichment will shed light on the dynamics of C retention and translocation for each species.

Further, we hypothesize that pulse crops in intercropping rely on biological N fixation for at least some portion of their N requirements. As such, we expect to see labeled ^{15}N enter the roots of pulse crops and subsequently be transferred to the leaves to fulfill N demand from photosynthetic enzymes. Additionally, we expect that a portion of N fixed by pulse crop roots will be made available to nearby barley companion crops, resulting in an enrichment in ^{15}N of barley roots.

Materials and Methods

This research was conducted at the University of Idaho's Research and Extension Center in Aberdeen, Idaho. The experimental fields were planted first in April 2020, and the isotopic labeling campaign was carried out in late June 2020, during the vegetative growth stage of pulse crops, when demand for nutrients is expected to be highest.

Two isotopic labeling campaigns lasting three consecutive days each were carried out on experimental plots that were part of the experimental setup described in chapter 2. In each campaign, a pulse plant (pea or lentil) from each barley/pulse intercropped plot under the full irrigation water treatment was dual-labeled with ^{13}C and ^{15}N gas over three days (Figure 1.2). A total of 16 plants (8 per campaign) were labeled; labeled plants were selected randomly, and edge rows were excluded from selection to minimize edge effects.

For ^{13}C labeling, plant canopies were enclosed in transparent plastic cuvettes to contain the label and minimize the reduction of sunlight to the leaves. Cuvettes were secured around the stem at ground level: flexible rubber semicircles with openings for the stem and gas tubing (inlet/outlet) were fitted around the stem to form a circular "plug" around which the cuvettes could be fastened without damaging the stem. An aqueous solution of 0.2g of labeled 99 atom% ^{13}C-$CaCO_3$ in 4ml of DI water was contained in a glass jar with fittings for the inlet and outlet tubes leading to the chamber. ^{13}C-CO_2 was produced by injecting 4ml of H_3PO_4 into the solution through a septum in the jar lid; at each label introduction period, the labeled gas was pumped into the canopy at a rate of approximately 1 liter/minute with a battery-operated pump.

For ^{15}N labeling, gas diffusers were inserted 30cm into the soil on either side of the labeled plant, at approximately 45° from vertical to converge beneath the roots of the crop. Plastic sheeting was placed as a barrier on the soil surface to delay the outgassing of N_2 to the atmosphere. A 3-liter Tedlar bag containing 98 atom% ^{15}N-N_2 gas was connected to the gas diffusers via the battery operated pump. At each label introduction period, the screw valve connecting the Tedlar bag to the pump system was opened and approximately 0.6 liters of N_2 gas was pumped to the diffusers. The valve to the Tedlar bag was closed in between label introduction periods.

Each day, the label was introduced using the pump system in 5 increments, approximately 45 minutes apart. This allowed the period that the plants were exposed to the label to be extended, increasing the amount of label uptake.

Labeling was carried out over three days to further maximize the amount of label introduced to the system. Introducing large amounts of label increased the likelihood of being able to trace fluxes of nutrients, especially microbial biomarkers, despite intensive dilution as it moves through the ecosystem. The magnitude of fluxes such as C compounds from the leaves of pulse crops to soil microbes, or N compounds from pulse roots to barley roots is potentially extremely small; greater amounts of label increase the possibility of capturing these transfers with the isotope label. This extended label period compromises a precise time course analysis during the chase period; because the label was not introduced all at once, processes with different rates may be conflated and interfere with the interpretation of enrichment.

After the labeling period, samples of plant tissues and soil were taken at the following intervals to trace the course of the label during the "chase" period (Table 3.1). Leaf samples were clipped from

the terminal leaves of labeled pulse crops; approximately 2 leaves were removed at each sample time to minimize damage to the plant.

Table 3.1: Samples collected at each sample interval during the chase period. Leaves and PLFA (soil) samples were collected at each time point; roots were sampled less frequently to minimize damage to the labeled plant that could impact survival and alter allocation strategies.

Time	Barley Root	Pulse Root	Pulse Leaf	PLFA
+ 0 (next day)		X	X	X
+ 1 hour			X	X
+ 8 hours			X	X
+ 24 hours	X	X	X	X
+ 2 days	X		X	X
+ 6 days	X	X	X	X
+ 14 days		X	X	X

Root samples for both pulse and barley crops were obtained from soil cores taken to ~8cm in the immediate vicinity of the sampled crop. The physical distinction of potentially intermixed pulse and barley roots was not possible, so the likelihood of roots being from the target crop only was maximized by sourcing samples from as close to the stem as possible. For analysis, root samples were considered to be exclusively from the target crop. Barley root samples were taken from plants on either side of the labeled pulse crop and were not combined, resulting in n=16 replicates for each sample time. Soil for PLFA was obtained from separate soil samples to ~8cm and was promptly frozen, then freeze-dried for storage prior to analysis (Quideau et al., 2016).

Analysis

Leaf samples were prepared by oven drying at 50°C until a constant mass was achieved, then homogenized to a fine powder using a ball mill (Spex 8000D Mixer/Mill). Root samples were separated by hand from air-dried soil by gently crushing aggregates and using a sieve to separate root material from soil, then homogenized by milling or cutting. Prepared samples (0.6-0.8mg) were weighed into 6x4mm tin capsules for isotope analysis using EA-IRMS (Costech ECS 4010 CHNSO analyzer, ThermoFisher Delta V Advantage IRMS) to obtain $\delta^{13}C$ and $\delta^{15}N$ values.

Soil samples for PLFA were freeze-dried prior to analysis, then extracted following a modified one-phase Bligh/Dyer method (Quideau et al., 2016). In short, lipids are extracted from the soil and

separated into classes by polarity using silica solid phase extraction columns to isolate the phospholipids. A subset of labeled PLFA samples was sent to the University of California, Davis Stable Isotope Facility for compound-specific isotope analysis, allowing tracing of the label into specific fatty acids. Identified fatty acid methyl esters (FAMEs) were aggregated into microbial groups for more meaningful interpretation (Supplemental Table 3.4).

Data from the two labeling campaigns were combined, increasing replicates to n = 8 for each chase sample period. For barley root C and N only, chase values were normalized by subtracting the average day 0 value from all subsequent values, allowing visualization of the trend in label over time. This also allows relative comparison of trends between different pools which otherwise may have been masked by different unlabeled/natural abundance values.

Isotope data for plant tissue samples are presented in delta notation (‰) Vienna-PeeDee Belemnite (VPDB) and air (AIR) were used as reference standards used to calculate $\delta^{13}C$ and $\delta^{15}N$, respectively. In-house standards were used for calibration and quantification of internal precision. We used an in-house sediment and leaf standard for drift and linearity corrections. The internal precision based on repeated standard analysis was 0.3‰ for C and 0.5‰ for N. Delta values are calculated as the ratio of heavy to light isotopes in the sample to the same ratio of the standard material (Equation 3.1) and are expressed in permil units.

$$\delta^{13}\mathrm{C} = \left(\frac{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)_{\mathrm{sample}}}{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)_{\mathrm{standard}}} - 1 \right) \times 1000\ ‰ \qquad (3.1)$$

Microbial ^{13}C uptake is expressed in atom percent excess ^{13}C of the microbial PLFAs (µg C kg^{-1}) using Equation 3.2 (Fuchslueger, Bahn, Fritz, Hasibeder, & Richter, 2014):

$$\left(\frac{atom\%_{sample} - atom\%_{NA} * Biomass\ [\mu g\ C]}{100} \right) * 1000\ (g) \qquad (3.2)$$

where $atom\%_{sample}$ is the atom% of the sample taken during the chase period, $atom\%_{NA}$ is the atom% of the PLFA sample prior to labeling, and Biomass is the mass of PLFA C. Atom percent notation is used for this pool as it is an exact method to quantify relatively small differences (Hayes, 2004).

Results

$\delta^{13}C$

Over the course of the chase period, ^{13}C label was detected in pulse leaves and roots (Figure 3.1), and soil microbial biomass (Figure 3.2). Initial $\delta^{13}C$ and $\delta^{15}N$ values for all sampled pools are reported in Supplemental Table 3.1.

Pulse leaf $\delta^{13}C$ values for both crops fluctuate for the first two days of the chase period, indicating fixed C was exiting and re-entering the leaves, before exhibiting opposite trends. Lentil leaves reach a maximum enrichment at the second sample period (607.5‰), then enrichment decreases for the remainder of the chase period to a minimum value of 287.5‰. Pea leaves have a similar initial $\delta^{13}C$ value to lentil leaves at day 0 (467.9‰ for peas, 494.9‰ for lentils), drop to a minimum enrichment of 158‰ on day 4, then slowly increase over the chase period to 410.5‰ by day 14.

By the end of the chase period, leaf tissues for both were depleted as compared to initial values indicating that overall, not all fixed carbon remained in the leaf tissue, but was either lost to respiration or allocated elsewhere.

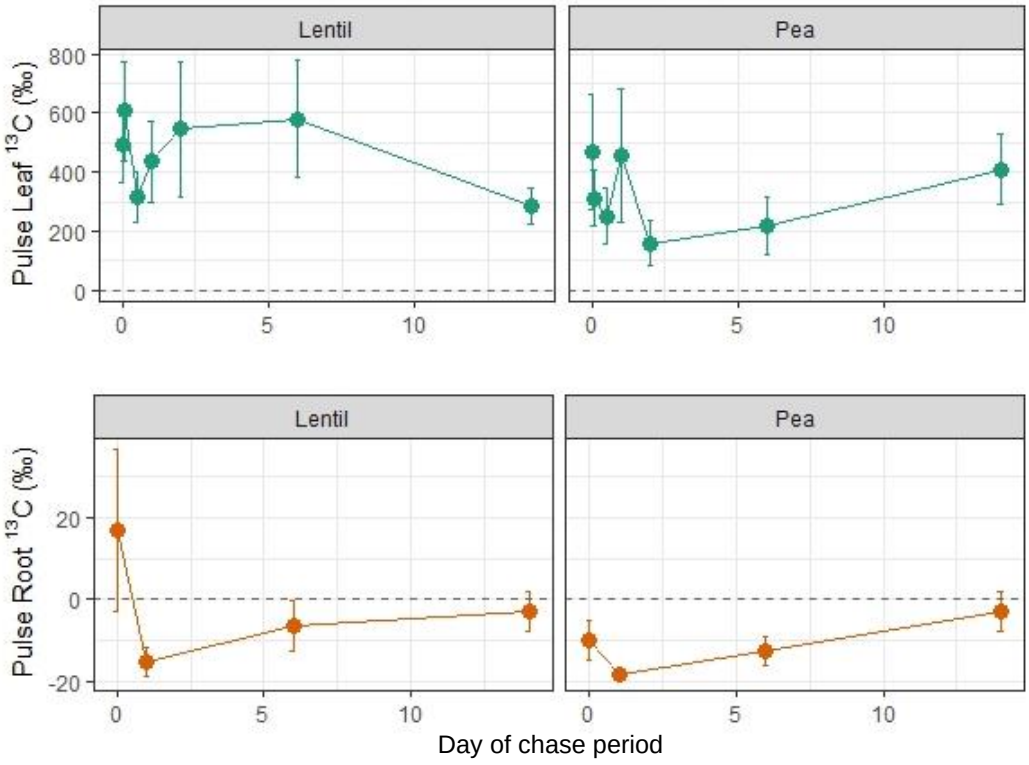

Figure 3.1: Raw (non-normalized) C isotope values for leaves (top panel) and roots (bottom panel) of pulse crops during the chase sampling period after 3 days of labeling. Greater relative depletion of both lentil tissues by the end of the chase period suggests faster rates of C transfer than in pea tissues. Mean and standard error values reported in supplemental table 2.

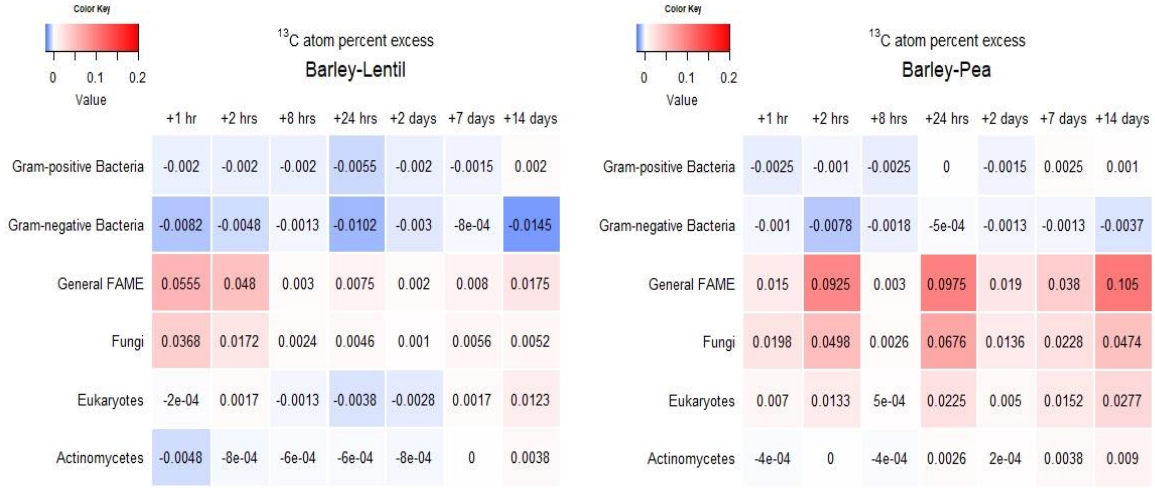

Figure 3.2: Heat map showing ^{13}C enrichment in atom %-excess of various soil microbial groups at barley-lentil (left) and barley-pea (right) intercropping sites. General FAME (fatty acid methyl ester), fungi, and eukaryote groups assimilated the most labeled C across both crop types during the chase period. Depleted (blue) values could indicate maximum uptake of ^{13}C during the label period, then a gradual depletion during the chase period. Enrichment values are as compared to samples taken prior to labeling.

Heat maps (Figure 3.2) show that ^{13}C fixed in the leaves of pulse crops was transferred to and mineralized by soil microbial communities within the chase period. For both pulse types, general FAME and fungi microbial groups took up the most label; for peas, eukaryotes were also enriched. In these groups for the pea plots, enrichment spiked at +2 hours, +24 hours, and +14 days. For lentils, fungi and general FAME groups were most enriched at the first two sampling times (+1 hour, +2 hours), with an overall depletion for the remainder of the chase period. Gram-negative bacteria for both crop treatments had the most depleted values, indicating ^{13}C label had been taken up prior to the beginning of chase sampling and was rapidly turned over from this group over time.

Overall, the microbial communities associated with pea crops were more enriched than those growing with lentil.

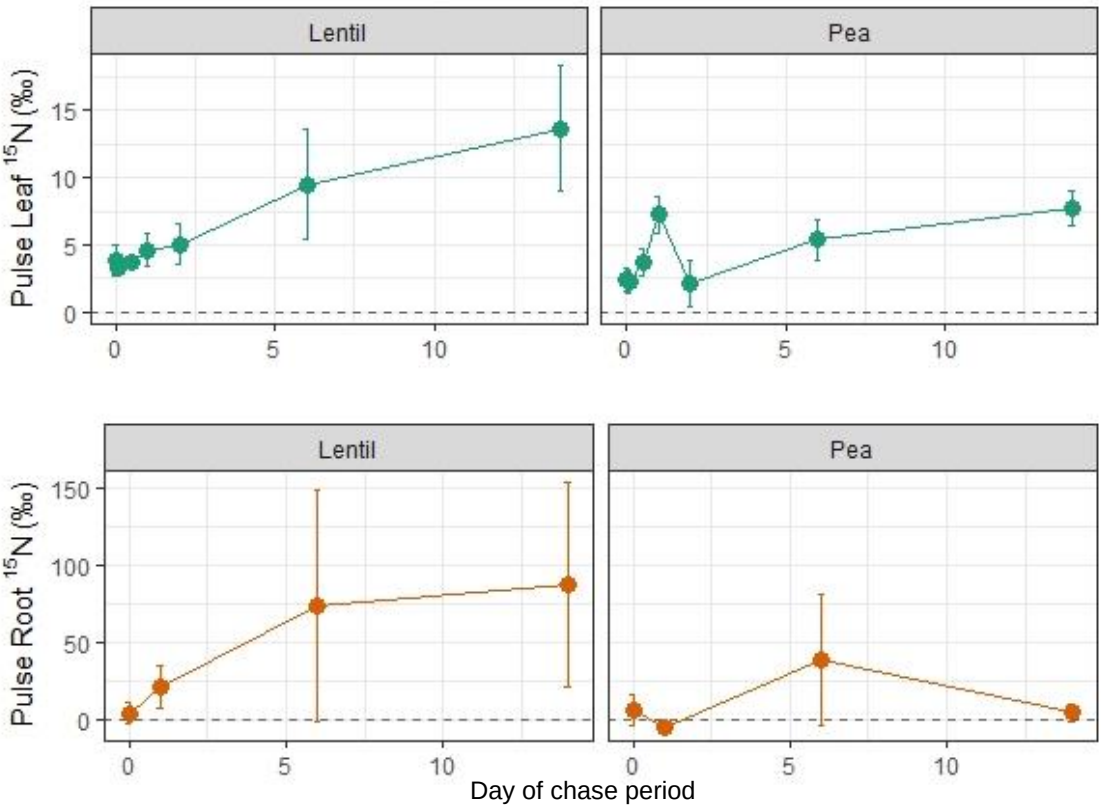

Figure 3.3: Raw (non-normalized) ^{15}N values of pulse crop leaves (top panel) and roots (bottom panel). Leaves of both crops are enriched over the course of the chase period, indicating transfer of fixed N from the roots. Lentil tissues took up more N label than pea tissues, indicating greater rates of biological nitrogen fixation. Mean and standard error values reported in Supplemental Table 2.

$\delta^{15}N$

Leaf tissues from both crops were progressively enriched in δ^{15}N over the course of the chase period, indicating N fixed in the roots was rapidly transferred to the leaves (Figure 3.3). However, not all of the N fixed in the roots was transferred to pulse leaves: leaf enrichment is roughly an order of magnitude smaller than root enrichment for both crops.

Maximum enrichment of lentil tissues was greater than that of pea for both leaves and roots, which indicates greater BNF in lentil crops. Trends in lentil root and leaf δ^{15}N enrichment are closely coupled over the course of the chase period, with no noticeable delay between the roots and leaves. Maximum enrichment for lentil roots was 87.7‰, and for leaves was 13.7‰.

Pea root maximum enrichment (38.9‰) occurred at chase day 6. Pea leaf maximum enrichment (7.7‰) occurred at chase day 14, but also spiked on chase day 1 (7.3‰). Though the absolute

magnitude of enrichment of pea leaves was less than lentil leaves, maximum pea leaf enrichment was 19.8% of maximum root enrichment, slightly greater than for lentils (15.6%).

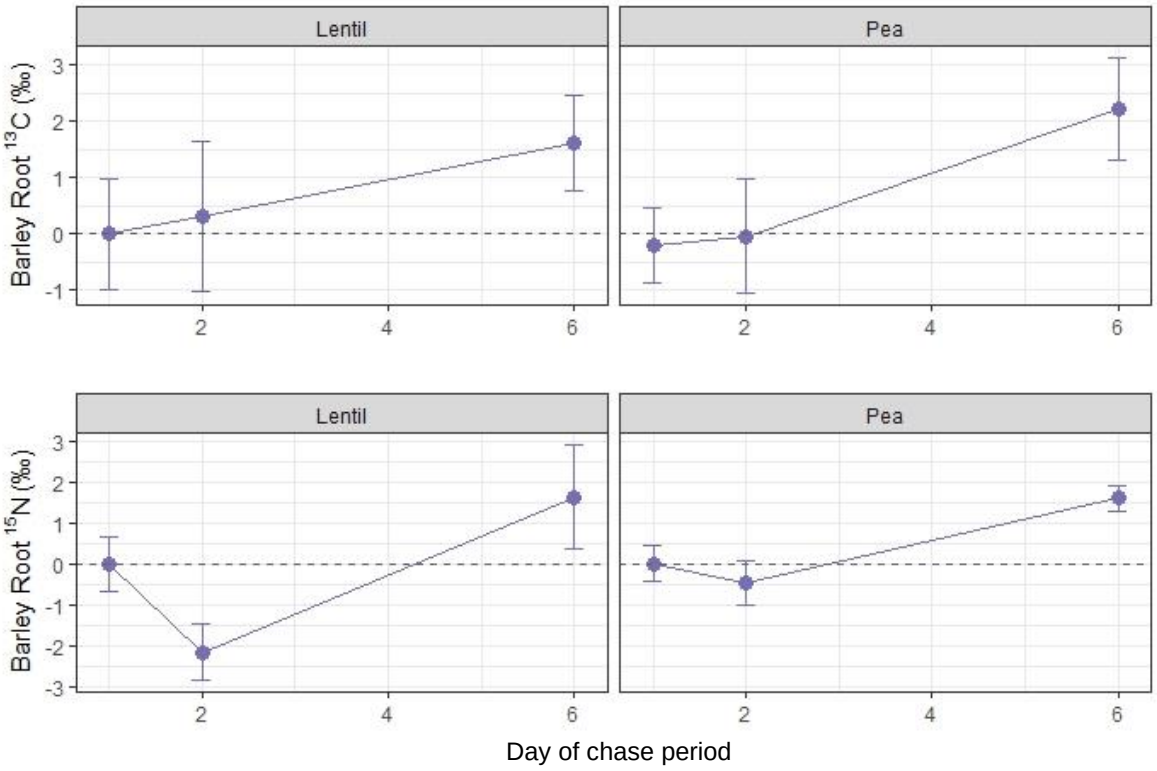

Figure 3.4: Normalized ^{13}C (top) and ^{15}N (bottom) values of barley roots from lentil (left panel) and pea (right panel) intercropping plots. Data normalized by values at the first sampling period (Supplemental Table 2), allowing clarification of enrichment patterns despite unique starting values for ^{13}C and ^{15}N. Enrichment of both C and N isotopes suggests that both nutrients fixed by pulse roots were made available for uptake by barley.

Barley roots from both lentil and pea intercropping plots were slightly enriched in ^{15}N and ^{13}C within the chase period, indicating C and N fixed by pulse crops were made available to adjacent barley crops (Figure 3.4).

Barley root samples were only taken at 3 points during the chase period (Table 3.1), and the magnitude of label enrichment in both δ^{13}C and δ^{15}N was small. To clarify patterns of enrichment during the chase period, the data in figure 3.4 are normalized by subtracting the isotope value of the first chase sample from the subsequent sample values. This allows direct comparison of relative isotope enrichment between ^{13}C and ^{15}N isotopes that would otherwise be obscured by very different natural abundance ranges for root tissues.

The decrease below baseline at sample day 2 for both crops suggests that some labeled N was already present in the root tissue at the time of day 0 sampling. While the three-day labeling period precludes

precise conclusions regarding timing, this does suggest that transfer from pulse roots to barley roots occurred within 72 hours (the time between label first being introduced and day 0 chase sampling).

Additionally, it is impossible to measure the isotopic values for precisely the same tissue every time, as sampling is destructive. Heterogeneous distribution of label among the overall tissue pool (in this case, barley roots) allows for the possibility of samples varying in enrichment purely by chance. To minimize this variability, we were careful to extract as much root biomass from soil samples as possible and homogenize all samples thoroughly before analysis. Standard errors are fairly small relative to the magnitude of enrichment, which lends support to the observed pattern of enrichment being experimentally derived rather than a sampling artifact.

Overall, we found that ^{13}C label traveled from the canopy of pulse crops, where it was fixed, to the roots of both pulse and barley crops and into the soil microbial biomass. Similarly, ^{15}N label was transferred from its origin at the pulse roots to the leaves of the pulse crop and to adjacent barley roots.

Discussion

Species selection

Pulses are intercropped with cereal crops to confer benefits including increasing N availability, C inputs, and microbial community diversity. Our results indicate that to realize these benefits, pulse crop species selection matters—not all pulses are created equal. The differences in nutrient cycling strategies may be inherent to the pulse species or elicited in intercropping because of competition with the companion crop. In this study, lentil and pea plants obtained and allocated both C and N in unique ways, which impacts availability to soil microbial communities and the barley companion crop. Barley biomass yields when intercropped with both pea and lentil decreased significantly ($p<0.05$), though not to the same extent as pulse biomass yields, likely due to reduced competition as well as beneficial interactions with pulses. Pulse species may primarily serve as advantageous inputs to barley in intercropping systems; pulse yields significantly decreased by an average of 86% ($p<0.001$) when intercropped.

Figure interpretation

In tissues where the label is present at chase day zero (roots for N and leaves for C), we would expect to see a negative slope of label enrichment from chase day zero, indicating decreasing amount of label as it is transferred out of that tissue to other pools. In pools where the label is not present at chase day zero, we would expect to see an overall positive slope from chase day zero, indicating the label is

entering that pool from where it was fixed. These general patterns are complicated somewhat by the nature of nutrient transfer, which can be bidirectional in nature (Aronoff, 1955; Biddulph & Cory, 1960; Jones, Martin, & Porter, 1959). Label that enters a tissue or pool may not remain and can be subsequently transferred back to the source or to another pool entirely, resulting in changing patterns in enrichment or depletion compared to chase day zero across the label period for various pools.

Additionally, some variability between samples of the same type is expected due to potentially unequal uptake and distribution of label across the entire plant canopy or roots. Removing tissues from the plant for sampling means that repeat measures of the exact same leaf or root are impossible, resulting in some heterogeneity. However, changes in the isotope values on the scale of tens or hundreds of permil (as in Figure 3.1) are likely much larger than this variability, allowing for interpretation of the patterns as plant-driven changes and not sampling artefacts.

C uptake

Our results demonstrate that pulse plants serve as a conduit connecting the atmosphere, soil, and microbial communities and can transfer C among these pools within a period of days. Within the plant, C translocation largely serves to support growth of new tissues. Mature leaves are a C source that allocate fixed C to sinks such as young leaves, seeds, flowers, and roots (Schaefer, Kier, & Stejskal, 1980; Turgeon, 1989). The magnitude of transfer of fixed C compounds increases with time, but prior isotope labeling research has indicated that transfer from sources to sinks begins immediately after C fixation (Brüggemann et al., 2011; Kayler, Keitel, Jansen, & Gessler, 2017; von Rein et al., 2016).

Overall, lentils have a more direct sink to the roots than peas, which appear to allocate C to additional (unsampled) pools (Figure 3.1). Different growth stages between the two crop species could drive these differences in allocation if C is translocated to reproductive tissues in pea rather than roots.

Within root tissues, both species prior to labeling have $\delta^{13}C$ values typical for C3 plants at -25.0‰ and -23.7‰, respectively (data not shown). After labeling, lentil roots are enriched to a greater degree than pea roots (19.9‰ and 4.9‰ respectively on chase day zero), demonstrating greater sink strength for lentil roots.

For lentils, the large spike in root $\delta^{13}C$ enrichment followed by a drop back to nearly baseline (-15.4‰) suggests that lentil leaves initially transferred a greater amount of fixed C to the roots, but this C was quickly either translocated back to the leaves or out into the soil. The slow enrichment of lentil roots for the remainder of the chase period coupled with the depletion of leaves indicates after

the initial flux, lentil leaves continue to function as a consistent “slow-release” source of fixed C to roots.

Within peas, $\delta^{13}C$ enrichment of leaves from chase day 2-14 is mirrored in the roots. This simultaneous enrichment of both sampled pools suggests a shift in plant source–sink dynamics. During a transition from vegetative to reproductive growth stages we can expect a change in the allocation of photosynthates, wherein C is allocated to reproductive sinks and the sink strength to root growth is dampened (Brüggemann et al., 2011).

Belowground communities

The connection of pulse crops to belowground communities is mediated through root exudates containing simple C substrates and secondary metabolites (Huang et al., 2014). These compounds provide substrate for growth and facilitate associations between plants and microbes, including that between legumes and nitrogen-fixing *Rhizobium* species (Broeckling, Broz, Bergelson, Manter, & Vivanco, 2008).

Within this study, microbial uptake of plant-sourced C varied between pulse crop species. Microbial PLFAs in barley-pea (BP) intercropping plots were more enriched in $\delta^{13}C$ (red cells) both in terms of magnitude and label persistence than PLFAs in the barley-lentil (BL) plots (Figure 3.2). This indicates that pea crops have a more “open” C cycle, retaining less C within the plant and providing more C-rich root exudates to the rhizosphere than lentils.

Enrichment of various microbial groups indicates activity, which allows inferences about the processes occurring in the soil. Enrichment was observed primarily in four groups: fungi, general FAME, eukaryotes, and gram-negative bacteria.

Fungi

Soil fungal communities are influenced by aboveground plant species, and in return provide feedback on plant growth through processes such as N fixation, pathogen defense, and soil organic matter stabilization (Frac et al., 2018). Two PLFAs (c18:1ω9c, c18:2ω6c/6t) were used to indicate the fungal community. The pronounced enrichment of this group indicates an active soil fungal community taking up plant-provided C. In this system, arbuscular mycorrhizal fungi (AMF) may be mediating the transfer of nutrients from pulse to nearby companion crops (Frac et al., 2018; Lesuffleur et al., 2013). The $\delta^{13}C$ enrichment of fungi paired with the transfer of both C and N label to barley roots (Figure 3.4) provides further support for the hypothesis of fungi-mediated nutrient transfer occurring between pulses and barley.

General FAME

Four saturated PLFAs (c14:0, c16:0, c17:0, c18:0) were assigned to the general FAME group. These biomarkers occur in several microbial groups or may be derived from plant compounds, leading to their non-specific categorization (Zosso & Wiesenberg, 2021). Relatively higher label uptake in this group may be a result of greater biomass; sources of these fatty acids are likely to occur more commonly in the soil than fatty acids linked to specific microbial groups.

Eukaryotes

Eukaryotes (c20:2ω6, c20:5ω3, c22:6ω3) represented the smallest amount of biomass of any group in our samples (data not shown), but eukaryotic microorganisms are often abundant in soils (Zhao et al., 2018). Protists—eukaryotes that are not otherwise classified as plants, animals, or fungi—are a diverse soil microbial group commonly known to consume other soil microbes like bacteria (Geisen et al., 2018). It's likely that the relatively small enrichment of eukaryotes in this study can be explained by this predation: the consumption of other microbes that have taken up ^{13}C label leads to subsequent, though diluted, ^{13}C enrichment of the eukaryotes.

Gram-Negative Bacteria

For both crop treatments, Gram-negative bacteria (c14:0 2-OH, c14:0 3-OH, c16:1ω9c/7t, c17:1ω6c, c18:1ω9t/7c, c18:1ω7t/5c) was the most depleted group. Nitrogen-fixing rhizobia bacteria fall into this category, so ^{13}C enrichment, indicating that the N-fixing bacteria are active and therefore require C compounds from the plant, was expected. The notable lack of ^{13}C label uptake may be explained by the endosymbiotic relationship of rhizobia, which live predominantly within the roots of their host plants, and are not abundant as free-living soil microbes (Schmidt, Bakole, & Bohlool, 1968). As such, other gram-negative species are likely to be the dominant species present in the PLFA sample. The relative depletion from T0 may further suggest that labeled C was taken up and cycled very rapidly, resulting in a net loss of label over the chase period.

The detection of labeled C in the microbial communities within a period of 6 days demonstrates the extensive and rapid connectivity of various pools mediated by plants, which has previously been observed (Tavi et al., 2013). The C allocation strategies between lentils and peas differed, but these differences did not correlate with significant differences in pulse production; intercropped lentil and pea produced 15.6% and 16.8% of sole crop biomass yields, respectively (Supplemental Table 3.3). These results suggest a more open flow of C in pea-cropping systems as compared to lentils, which

may retain more of the fixed C within the tissues of the plant rather than releasing it to the roots or soil for microbial uptake.

N fixation

Leguminous crops have varying abilities to regulate N-fixation in response to changing soil nutrient availability, with some able to decrease the rate of this costly process when N is sufficient, and others maintaining a relatively constant rate of fixation (Dovrat et al., 2020). Greater ^{15}N enrichment of lentil roots as compared to pea roots (Figure 3.3) demonstrates that lentils were more reliant on BFN compared to peas. This combined with smaller decreases in soil N over the growing season for crop treatments with lentil (Chapter 2) than pea suggest that lentils lack the ability to down-regulate N-fixation in response to soil nutrient status, and instead have an obligate fixation rate.

The decrease in ^{15}N label magnitude of >80% as it is translocated from root to leaf for both crops suggests that leaves are not the exclusive sink for fixed N. Most plant N is allocated to leaves, fruits, and seeds, and the balance between these pools may indicate the priorities of the growth stage the crop is in (i.e., vegetative growth vs reproduction) (Weiner, Campbell, Pino, & Echarte, 2009). Relatively greater allocation of N to leaves in lentils than peas (as with C) further supports the idea that peas may have been entering a reproductive growth stage during the chase period and allocating nutrients to reproductive tissues.

The presence of label in barley roots reveals transfer of N from pulse crops to companion barley, which is important from an agronomic perspective (Figure 3.4). Notably, peas were shown to transfer relatively more N to their companion crops than lentil; barley roots growing in both intercropping systems were approximately equal in the magnitude of maximum ^{15}N enrichment despite differences in the enrichment of lentil and pulse roots. This is further evidence that peas are less conservative in resource retention than lentils and allow more open nutrient cycling and greater allocation to pools outside of the pulse crop itself.

Transferred N is taken up by companion crops either through direct root contact, water-facilitated transport of soluble forms of N (NO_3^-), or through direct transfer by arbuscular mycorrhizal fungi (AMF) symbionts associated with the roots of both crops. As previously noted, the presence of ^{13}C and ^{15}N label in barley roots suggests the transfer of plant-available N from pulse roots we observed is mediated by AMF. These symbionts rely on plant-derived organic C and have been shown to facilitate the transfer of inorganic N between cereal and pulse crops (Johansen & Jensen, 1996; Tomè, Tagliavini, & Scandellari, 2015). This would explain the presence of both C and N in barley roots: N

as the intended transfer nutrient, and C as a "byproduct" of fueling the transfer itself. Fungal community enrichment observed in both crop strategies (Figure 3.2) additionally supports this hypothesis. It is possible that the label presence is a sampling artifact from pulse root material being collected along with barley root in the sampling (Figure 3.4), but precautions taken to isolate each crop species' roots (see methods) make this unlikely.

Importance of species

Clearly, pulse crop species consideration matters when intercropping. Lentils and peas differ in the amount of N fixation and strategies of nutrient allocation both within and beyond the plant. These differences were not expressed noticeably in the biomass yield of intercropped pulses (15.6% and 16.8% of sole crop yield for lentils and peas, respectively), but may explain slightly larger differences in the biomass yield of the companion barley crop (64.2% and 69.5% of sole crop for barley intercropped with lentils and peas, respectively) (Supplemental Table 3.3). It is possible that the more open nutrient cycling observed in peas supports companion crops to a greater extent than lentils. Although lentils were found to rely more heavily on N fixation than peas, competition for soil N by peas did not decrease barley biomass yields in comparison to barley grown with lentils.

Future Directions

The results of this work represent a first step toward assessing nutrient cycling in a pulse intercropping system, and how it may influence soil health. In addition to the observations drawn in this chapter, the results are further valuable as they demonstrate that the novel gaseous dual-label introduction system was effective at delivering both ^{15}N and ^{13}C label to the pulse crop and can be used for continued research. Future efforts to conduct labeling of monocropped pulses as well as crop treatments in deficit irrigation will greatly enhance our understanding of how intercropping and water availability influence nutrient cycling and allocation in pea and lentil plants. Sampling of the entire labeled plant in future work, as opposed to only sampling select tissues, may also help elucidate sources and sinks of both C and N, and close the knowledge gap on the fate of assimilated label. Further, although transfer of N from pulse crops to adjacent barley was observed, quantification of this transfer will be a vital next step in assessing the feasibility to producers of intercropping as a substitute for rotation in terms of N supply to barley.

Conclusion

This study sought to understand the connectivity between companion pulse and barley crops and the soil ecosystem in an intercropping system with regard to C and N cycling. By using stable isotope

tracing, we were able to visualize how these elements are fixed by plant tissues and allocated to various pools over two weeks after introducing isotopic label to the system.

Carbon and nitrogen cycling are intricately linked in terrestrial ecosystems. Limited N availability is the most common constraint on photosynthetic C fixation and plant growth; C-rich photosynthates provide fuel not only for plants but for many organisms including symbiotic N-fixing bacteria associated with pulse crop roots. The availability of N plays an important role in regulating the productivity of ecosystems, which impacts the yield and profit in agricultural systems.

We hypothesized that C fixed in pulse leaves would be translocated to pulse roots and further to soil microbial communities via root exudates. Further, we expected that nitrogen fixed to a plant-available form by symbiotic bacteria associated with pulse roots would be translocated to pulse leaves to increase photosynthetic capacity as well as to companion barley crop roots. Isotope label was detected in every sampled pool within the duration of the 14-day chase period, supporting our hypotheses of rapid and extensive pulse-crop mediated transfer of C and N and indicating the real-time connectivity of companion pulse and barley crops.

The results of this work suggest that the pulse crops species we studied allocate resources differently in intercropping systems and are not directly interchangeable. Though both peas and lentils fixed N that was then available for uptake by companion barley crops, the magnitude of BNF and within-plant allocation of N varied with lentils fixing more N overall but directing relatively less of it to the leaves. Similarly, both crops made fixed C available to soil microbial communities via root exudates, but greater enrichment of microbial groups associated with pea crops suggests a more open C cycle than for lentils. Further research to compare nutrient cycling of pulses grown alone to the intercropped pulses studied here could help to elucidate these dynamics and understand the obligate or facultative nature of N fixation and nutrient cycling among pea and lentil crops.

References

Andrews, S. S., Karlen, D. L., & Cambardella, C. a. (2004). The Soil Management Assessment Framework: A quantitative soil quality evaluation method. *Soil Science Society of America Journal*, *68*(6), 1945–1962.

Aronoff, S. (1955). Translocation from Soybean Leaves, II. *Plant Physiology*, *30*(2), 184–185. https://doi.org/10.1104/pp.30.2.184

Avice, J. C., Ourry, A., Lemaire, G., & Boucaud, J. (1996). Nitrogen and carbon flows estimated by 15N and 13C pulse-chase labeling during regrowth of Alfalfa. *Plant Physiology*, *112*(1), 281–290. https://doi.org/10.1104/pp.112.1.281

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microbial Ecology*, *45*(4), 373–383. https://doi.org/10.1007/s00248-003-2002-y

Bedoussac, L., Journet, E. P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E. S., … Justes, E. (2015). Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agronomy for Sustainable Development*, *35*(3), 911–935. https://doi.org/10.1007/s13593-014-0277-7

Bennett, L. T., Mele, P. M., Annett, S., & Kasel, S. (2010). Examining links between soil management, soil health, and public benefits in agricultural landscapes: An Australian perspective. *Agriculture, Ecosystems and Environment*, *139*(1–2), 1–12. https://doi.org/10.1016/j.agee.2010.06.017

Biddulph, O., & Cory, R. (1960). Demonstration of Two Translocation Mechanisms in Studies of Bidirectional Movement. *Plant Physiology*, *35*(5), 689–695. https://doi.org/10.1104/pp.35.5.689

Bossio, D. A., & Scow, K. M. (1998). Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology*, Vol. 35, pp. 265–278. https://doi.org/10.1007/s002489900082

Bossio, D. A., Scow, K. M., Gunapala, N., & Graham, K. J. (1998). Determinants of Soil Microbial Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty Acid Profiles. *Microbial Ecology*, (36), 1–12.

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology*, *74*(3), 738–744. https://doi.org/10.1128/AEM.02188-07

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., … White, P. J. (2015). Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, Vol. 206, pp. 107–117. https://doi.org/10.1111/nph.13132

Brüggemann, N., Gessler, A., Kayler, Z., Keel, S. G., Badeck, F., Barthel, M., … Bahn, M. (2011). Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: A review. *Biogeosciences*, *8*(11), 3457–3489. https://doi.org/10.5194/bg-8-3457-2011

Cardoso, E. J. B. N., Vasconcellos, R. L. F., Bini, D., Miyauchi, M. Y. H., dos Santos, C. A., Alves,

P. R. L., … Nogueira, M. A. (2013). Soil health: Looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola*, *70*(4), 274–289. https://doi.org/10.1590/S0103-90162013000400009

Carter, M. S., & Ambus, P. (2006). Biologically fixed N2 as a source for N2O production in a grass-clover mixture, measured by 15N2. *Nutrient Cycling in Agroecosystems*, *74*(1), 13–26. https://doi.org/10.1007/s10705-005-4111-0

Chalk, P. M., He, J. Z., Peoples, M. B., & Chen, D. (2017). 15N2 as a tracer of biological N2 fixation: A 75-year retrospective. *Soil Biology and Biochemistry*, *106*, 36–50. https://doi.org/10.1016/j.soilbio.2016.12.010

Chapagain, T., & Riseman, A. (2014). Barley-pea intercropping: Effects on land productivity, carbon and nitrogen transformations. *Field Crops Research*, *166*(September), 18–25. https://doi.org/10.1016/j.fcr.2014.06.014

Cherkasov, N., Ibhadon, A. O., & Fitzpatrick, P. (2015). A review of the existing and alternative methods for greener nitrogen fixation. *Chemical Engineering and Processing: Process Intensification*, Vol. 90, pp. 24–33. https://doi.org/10.1016/j.cep.2015.02.004

Clermont-Dauphin, C., Dissataporn, C., Suvannang, N., Pongwichian, P., Maeght, J. luc, Hammecker, C., & Jourdan, C. (2018). Intercrops improve the drought resistance of young rubber trees. *Agronomy for Sustainable Development*, Vol. 38. https://doi.org/10.1007/s13593-018-0537-z

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, *85*(3), 235–252. https://doi.org/10.1007/s10533-007-9132-0

Corre-Hellou, G., Fustec, J., & Crozat, Y. (2006). Interspecific competition for soil N and its interaction with N2 fixation, leaf expansion and crop growth in pea-barley intercrops. *Plant and Soil*, *282*(1–2), 195–208. https://doi.org/10.1007/s11104-005-5777-4

Coskun, D., Britto, D. T., & Kronzucker, H. J. (2016). Nutrient constraints on terrestrial carbon fixation: The role of nitrogen. *Journal of Plant Physiology*, *203*, 95–109. https://doi.org/10.1016/j.jplph.2016.05.016

Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Denef, K., & Paul, E. (2013). The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. https://doi.org/10.1111/gcb.12113

Cox, H. W., Kelly, R. M., & Strong, W. M. (2010). Pulse crops in rotation with cereals can be a profitable alternative to nitrogen fertiliser in central Queensland. *Crop and Pasture Science*, *61*(9), 752–762. https://doi.org/10.1071/CP09352

Culman, S., Freeman, M., & Snapp, S. (2012). Procedure for the Determination of Permanganate Oxidizable Carbon. *KBS POXC Protocol – Last Revised*, *1*, 1–5.

Dovrat, G., Bakhshian, H., Masci, T., & Sheffer, E. (2020). The nitrogen economic spectrum of legume stoichiometry and fixation strategy. *New Phytologist*, Vol. 227, pp. 365–375. https://doi.org/10.1111/nph.16543

Drake, D. C. (2011). Invasive legumes fix N2 at high rates in riparian areas of an N-saturated,

agricultural catchment. *Journal of Ecology*, *99*(2), 515–523. https://doi.org/10.1111/j.1365-2745.2010.01787.x

Economic Impacts of Climate Change on Agriculture in Idaho. (2018). *The Impact of Climate Change on Our Life: The Questions of Sustainability*, 179–195. https://doi.org/10.1007/978-981-10-7748-7_10

Farquhar, G. D., Fasham, M. J. R., Goulden, M. L., Heimann, M., Jaramillo, V. J., Kheshgi, H. S., … Wallace, D. W. R. (2001). The Carbon Cycle and Atmospheric Carbon Dioxide. In *IPCC, 2001: Climate Change 2001: The Scientific Basis.2*. https://doi.org/10.1029/eo067i015p00191

Frac, M., Hannula, S. E., Belka, M., & Jędryczka, M. (2018). Fungal biodiversity and their role in soil health. *Frontiers in Microbiology*, *9*(APR), 1–9. https://doi.org/10.3389/fmicb.2018.00707

Franzluebbers, A. J., Hons, F. M., & Zuberer, D. A. (1995). Tillage and Crop Effects on Seasonal Soil Carbon and Nitrogen Dynamics. *Soil Science Society of America Journal*, *59*(6), 1618–1624. https://doi.org/10.2136/sssaj1995.03615995005900060016x

Frostegard, A., & Baath, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, *22*(1–2), 59–65. https://doi.org/10.1007/bf00384433

Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R., & Richter, A. (2014). Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow. *New Phytologist*, Vol. 201, pp. 916–927. https://doi.org/10.1111/nph.12569

Fustec, J., Lesuffleur, F., Mahieu, S., & Cliquet, J. B. (2009). Nitrogen rhizodeposition of legumes. *Sustainable Agriculture*, *2*, 869–881. https://doi.org/10.1007/978-94-007-0394-0_38

Galloway, J., Dentener, F., Capone, D. G., Boyer, E. W., Howarth, R. ., Seitzinger, S., … Vorosmarty, C. J. (2004). Nitrogen Cycles: past, present, and future. *Biogeochemistry*, *70*, 153–226.

Gan, Y., Hamel, C., O'Donovan, J. T., Cutforth, H., Zentner, R. P., Campbell, C. A., … Poppy, L. (2015). Diversifying crop rotations with pulses enhances system productivity. *Scientific Reports*, *5*, 1–14. https://doi.org/10.1038/srep14625

Geisen, S., Mitchell, E. A. D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., … Lara, E. (2018). Soil protists: a fertile frontier in soil biology research. *FEMS Microbiology Reviews*, *42*(3), 293–323. https://doi.org/10.1093/femsre/fuy006

Glaze-Corcoran, S., Hashemi, M., Sadeghpour, A., Jahanzad, E., Keshavarz Afshar, R., Liu, X., & Herbert, S. J. (2020). Understanding intercropping to improve agricultural resiliency and environmental sustainability. *Advances in Agronomy*, Vol. 162, pp. 199–256. https://doi.org/10.1016/bs.agron.2020.02.004

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: Tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362–2371. https://doi.org/10.1002/jsfa.6577

Hauggaard-Nielsen, H., Ambus, P., & Jensen, E. S. (2001). Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops - A field study employing 32p technique.

Plant and Soil, *236*(1), 63–74. https://doi.org/10.1023/A:1011909414400

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., … Jensen, E. S. (2009). Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crops Research*, *113*(1), 64–71. https://doi.org/10.1016/j.fcr.2009.04.009

Hauggaard-Nielsen, H., & Jensen, E. S. (2005). Facilitative root interactions in intercrops. *Plant and Soil*, *274*(1–2), 237–250. https://doi.org/10.1007/s11104-004-1305-1

Hauggaard-Nielsen, Henrik, Ambus, P., & Jensen, E. S. (2001). Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research*, *70*, 101–109.

Hayes, J. M. (2004). Isotopic Mass Balance Equations. *Woods Hole Oceanographic Institution*, (September), 1–10. Retrieved from https://www.whoi.edu/cms/files/jhayes/2005/9/IsoCalcs30Sept04_5183.pdf%0Ahttp://www.google.com/search?client=safari&rls=en&q=isotopic+mass+balance+equations&ie=UTF-8&oe=UTF-8%5Cnpapers3://publication/uuid/690A8C6A-C245-4571-899D-D250AD3EA88A

Høgh-Jensen, H. (2006). The nitrogen transfer between plants: An important but difficult flux to quantify. *Plant and Soil*, *282*(1–2), 1–5. https://doi.org/10.1007/s11104-005-2613-9

Houghton, R. A. (2003). The Contemporary Carbon Cycle. In *Treatise on Geochemistry* (pp. 473–513).

Huang, X., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., & Vivanco, J. M. (2014). *Rhizosphere interactions: root exudates, microbes, and microbial communities 1*. *275*(February), 267–275.

IFA – Internationl Fertilizer Association. (2021). Public Summary Medium-Term Fertilizer Outlook 2021 – 2025. *Public Summary Medium-Term Fertilizer Outlook 2021 – 2025. IFA, 2021.*, *1*(August), 1–9. Retrieved from https://www.ifastat.org/

Jenkinson, D. S. (2001). The impact of humans on the nitrogen cycle, with focus on temperate arable agriculture. *Plant and Soil*, *228*(1), 3–15. https://doi.org/10.1023/A:1004870606003

Jensen, E. S. (1996). Grain yield, symbiotic N_2 fixation and interspecific competition for inorganic N in pea-barley intercrops. *Plant and Soil*, *182*(1), 25–38.

Jiang-shan, Z., Jian-fen, G., Guang-shui, C., & Wei, Q. (2005). Soil microbial biomass and its controls. *Journal of Forestry Research*, *16*(4), 327–330. https://doi.org/10.1007/bf02858201

Jiao, S., Wang, J., Wei, G., Chen, W., & Lu, Y. (2019). Dominant role of abundant rather than rare bacterial taxa in maintaining agro-soil microbiomes under environmental disturbances. *Chemosphere*, *235*, 248–259. https://doi.org/10.1016/j.chemosphere.2019.06.174

Johansen, A., & Jensen, E. S. (1996). Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology and Biochemistry*, *28*(1), 73–81. https://doi.org/10.1016/0038-0717(95)00117-4

Johnson, P. T. J., Townsend, A. R., Cleveland, C. C., Glibert, P. M., Howarth, R. W., Mckenzie, V. J., … Ward, M. H. (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. *Ecological Applications*, Vol. 20, pp. 16–29. https://doi.org/10.1890/08-0633.1

Jones, H., Martin, R. V., & Porter, H. K. (1959). Translocation of ^{14}Carbon in Tobacco following Assimilation of ^{14}Carbon Dioxide by a Single Leaf. *Annals of Botany*, *23*(92), 493–508.

Kayler, Z., Keitel, C., Jansen, K., & Gessler, A. (2017). Experimental evidence of two mechanisms coupling leaf-level C assimilation to rhizosphere CO2 release. *Environmental and Experimental Botany*, *135*, 21–26. https://doi.org/10.1016/j.envexpbot.2016.12.002

Keeling, C. D., Piper, S. C., Bacastow, R. B., Wahlen, M., Whorf, T. P., Heimann, M., & Meijer, H. A. (2005). Terrestrial Biosphere and Oceans from 1978 to 2000 : Observations and Carbon Cycle Implications. *A History of Atmospheric CO2 and Its Effects on Plants, Animals, and Ecosystems*, 83–113.

Koong, D., Benz, S., Johnson, B., Losh, D., Stebbins, E., & Reichert, J. (2020). 2020 Idaho Annual Statistical Bulletin. *USDA National Agricultural Statistics Service*.

Lal, R. (2014). Societal value of soil carbon. *Journal of Soil and Water Conservation*, *69*(6), 186A-192A. https://doi.org/10.2489/jswc.69.6.186A

Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability (Switzerland)*, Vol. 7, pp. 5875–5895. https://doi.org/10.3390/su7055875

LeBauer, D; Treseder, K. (2008). Nitrogen Limitation of Net Primary Productivity. *Ecology*, *89*(2), 371–379.

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., … Stott, D. E. (2015). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, *70*(1), 12A-18A. https://doi.org/10.2489/jswc.70.1.12A

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth and Environment*, Vol. 1, pp. 544–553. https://doi.org/10.1038/s43017-020-0080-8

Lesuffleur, F., Salon, C., Jeudy, C., & Cliquet, J. B. (2013). Use of a 15N2 labelling technique to estimate exudation by white clover and transfer to companion ryegrass of symbiotically fixed N. *Plant and Soil*, *369*(1–2), 187–197. https://doi.org/10.1007/s11104-012-1562-3

Li, C., Cano, A., Acosta-Martinez, V., Veum, K. S., & Moore-Kucera, J. (2020). A comparison between fatty acid methyl ester profiling methods (PLFA and EL-FAME) as soil health indicators. *Soil Science Society of America Journal*, *84*(4), 1153–1169. https://doi.org/10.1002/saj2.20118

Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. A., & Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia*, *147*(2), 280–290. https://doi.org/10.1007/s00442-005-0256-4

Lian, T., Mu, Y., Jin, J., Ma, Q., Cheng, Y., Cai, Z., & Nian, H. (2019). Impact of intercropping on the coupling between soil microbial community structure, activity, and nutrient-use efficiencies. *PeerJ*, *2019*(2), 1–18. https://doi.org/10.7717/peerj.6412

Lithourgidis, A. S., Dordas, C. A., Damalas, C. A., & Vlachostergios, D. N. (2011). Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop Science*, *5*(4), 396–410.

Lu, C., & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: Shifted hot spots and nutrient imbalance. *Earth System Science Data*, *9*(1),

181–192. https://doi.org/10.5194/essd-9-181-2017

Luo, X., Keenan, T. F., Chen, J. M., Croft, H., Colin Prentice, I., Smith, N. G., … Zhang, Y. (2021). Global variation in the fraction of leaf nitrogen allocated to photosynthesis. *Nature Communications*, *12*(1), 1–10. https://doi.org/10.1038/s41467-021-25163-9

Malik, A. A., Chowdhury, S., Schlager, V., Oliver, A., Puissant, J., Vazquez, P. G. M., … Gleixner, G. (2016). Soil fungal: Bacterial ratios are linked to altered carbon cycling. *Frontiers in Microbiology*, *7*(AUG), 1–11. https://doi.org/10.3389/fmicb.2016.01247

Mann, C., Lynch, D., Fillmore, S., & Mills, A. (2019). Relationships between field management, soil health, and microbial community composition. *Applied Soil Ecology*, *144*(December 2018), 12–21. https://doi.org/10.1016/j.apsoil.2019.06.012

Manninen, N., Soinne, H., Lemola, R., Hoikkala, L., & Turtola, E. (2018). Effects of agricultural land use on dissolved organic carbon and nitrogen in surface runoff and subsurface drainage. *Science of the Total Environment*, *618*, 1519–1528. https://doi.org/10.1016/j.scitotenv.2017.09.319

McConnell, M., Liefert, O., Williams, A., & Boline, A. (2022). Feed Outlook: January 2022. *UDSA Economic Research Service Situation and Outlook Report*, *FDS-22a*(January).

Menge, D. N. L., Levin, S. A., & Hedin, L. O. (2009). Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. *American Naturalist*, *174*(4), 465–477. https://doi.org/10.1086/605377

Menge, D. N. L., Wolf, A. A., & Funk, J. L. (2015). Diversity of nitrogen fixation strategies in Mediterranean legumes. *Nature Plants*, *1*(6), 1–5. https://doi.org/10.1038/nplants.2015.64

Millar, N., Urrea, A., Kahmark, K., Shcherbak, I., Robertson, G. P., & Ortiz-Monasterio, I. (2018). Nitrous oxide (N2O) flux responds exponentially to nitrogen fertilizer in irrigated wheat in the Yaqui Valley, Mexico. *Agriculture, Ecosystems and Environment*, *261*(April), 125–132. https://doi.org/10.1016/j.agee.2018.04.003

Miller, P. R., McConkey, B. G., Clayton, G. W., Brandt, S. A., Staricka, J. A., Johnston, A. M., … Neill, K. E. (2002). Pulse crop adaptation in the northern Great Plains. *Agronomy Journal*, *94*(2), 261–272. https://doi.org/10.2134/agronj2002.0261

Minchin, F. R., & Pate, J. S. (1973). The carbon balance of a legume and the functional economy of its root nodules. *Journal of Experimental Botany*, *24*(2), 259–271. https://doi.org/10.1093/jxb/24.2.259

Moebius-Clune, B. N., Moebius-Clune, D. J., Gugino, B. K., Idowu, O. J., Schindelbeck, R. R., Ristow, A. J., … Abawi, G. S. (2016). Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.1. In *Cornell University, Geneva, NY*.

Moore-Kucera, J., & Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. *Microbial Ecology*, *55*(3), 500–511. https://doi.org/10.1007/s00248-007-9295-1

Moyer-Henry, K. A., Burton, J. W., Israel, D. W., & Rufty, T. W. (2006). Nitrogen transfer between plants: A 15N natural abundance study with crop and weed species. *Plant and Soil*, *282*(1–2), 7–20. https://doi.org/10.1007/s11104-005-3081-y

National Agricultural Statistics Service, U. (2020). *Small Grains 2020 Summary*. (September).

Naylor, D., & Coleman-Derr, D. (2018). Drought stress and root-associated bacterial communities. *Frontiers in Plant Science*, *8*(January), 1–16. https://doi.org/10.3389/fpls.2017.02223

Norris, C. E., Bean, G. Mac, Cappellazzi, S. B., Cope, M., Greub, K. L. H., Liptzin, D., … Honeycutt, C. W. (2020). Introducing the North American project to evaluate soil health measurements. *Agronomy Journal*, Vol. 112, pp. 3195–3215. https://doi.org/10.1002/agj2.20234

Outlaw, J. L., Bryant, H. L., Raulston, J. M., Knapek, G. M., Herbst, B. K., & Fischer, B. L. (2022). *Economic Impact of Higher Fertilizer Prices on AFPC's Representative Crop Farms*.

Paynel, F., Murray, P. J., & Bernard Cliquet, J. (2001). Root exudates: A pathway for short-term N transfer from clover and ryegrass. *Plant and Soil*, *229*(2), 235–243. https://doi.org/10.1023/A:1004877214831

Peoples, M. B., Giller, K. E., Jensen, E. S., & Herridge, D. F. (2021). Quantifying country-to-global scale nitrogen fixation for grain legumes: I. Reliance on nitrogen fixation of soybean, groundnut and pulses. *Plant and Soil*, *469*(1–2), 1–14. https://doi.org/10.1007/s11104-021-05167-6

Pikaar, I., Matassa, S., Rabaey, K., Bodirsky, B. L., Popp, A., Herrero, M., & Verstraete, W. (2017). Microbes and the Next Nitrogen Revolution. *Environmental Science and Technology*, *51*(13), 7297–7303. https://doi.org/10.1021/acs.est.7b00916

Pirhofer-Walzl, K., Rasmussen, J., Høgh-Jensen, H., Eriksen, J., Søegaard, K., & Rasmussen, J. (2012). Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil*, *350*(1–2), 71–84. https://doi.org/10.1007/s11104-011-0882-z

Quideau, S. A., McIntosh, A. C. S., Norris, C. E., Lloret, E., Swallow, M. J. B., & Hannam, K. (2016). Extraction and analysis of microbial Phospholipid fatty acids in soils. *Journal of Visualized Experiments*, *2016*(114), 1–9. https://doi.org/10.3791/54360

Rasse, D. P., Rumpel, C., & Dignac, M. F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*(1–2), 341–356. https://doi.org/10.1007/s11104-004-0907-y

Renwick, L. L. R., Kimaro, A. A., Hafner, J. M., Rosenstock, T. S., & Gaudin, A. C. M. (2020). Maize-Pigeonpea Intercropping Outperforms Monocultures Under Drought. *Frontiers in Sustainable Food Systems*, *4*(December). https://doi.org/10.3389/fsufs.2020.562663

Rodriguez, C., Carlsson, G., Englund, J. E., Flöhr, A., Pelzer, E., Jeuffroy, M. H., … Jensen, E. S. (2020). Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. *European Journal of Agronomy*, *118*(July 2019). https://doi.org/10.1016/j.eja.2020.126077

Rousk, J., Brookes, P. C., & Bååth, E. (2010). The microbial PLFA composition as affected by pH in an arable soil. *Soil Biology and Biochemistry*, *42*(3), 516–520. https://doi.org/10.1016/j.soilbio.2009.11.026

Savci, S. (2012). Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia*, *1*(January), 287–292. https://doi.org/10.1016/j.apcbee.2012.03.047

Schaefer, J., Kier, L. D., & Stejskal, E. O. (1980). Characterization of Photorespiration in Intact Leaves Using 13 Carbon Dioxide Labeling . *Plant Physiology*, *65*(2), 254–259. https://doi.org/10.1104/pp.65.2.254

Schmidt, E. L., Bakole, R. O., & Bohlool, B. B. (1968). Fluorescent-antibody approach to study of

rhizobia in soil. *Journal of Bacteriology*, *95*(6), 1987–1992. https://doi.org/10.1128/jb.95.6.1987-1992.1968

Schulze, J. (2004). How are nitrogen fixation rates regulated in legumes? *Journal of Plant Nutrition and Soil Science*, *167*(2), 125–137. https://doi.org/10.1002/jpln.200320358

Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N 2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(25), 9199–9204. https://doi.org/10.1073/pnas.1322434111

Shestak, C. J., & Busse, M. D. (2005). Compaction Alters Physical but Not Biological Indices of Soil Health. *Soil Science Society of America Journal*, Vol. 69, p. 236. https://doi.org/10.2136/sssaj2005.0236

Smil, V. (1999). Nitrogen in crop production : An account of global flows adds. *Global Biogeochemical Cycles*, *13*(2), 647–662.

Stehfest, E., & Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems*, *74*, 207–228.

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., … Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment*, Vol. 164, pp. 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Strickland, M. S., & Rousk, J. (2010). Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, *42*(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Tavi, N. M., Martikainen, P. J., Lokko, K., Kontro, M., Wild, B., Richter, A., & Biasi, C. (2013). Linking microbial community structure and allocation of plant-derived carbon in an organic agricultural soil using 13CO2 pulse-chase labelling combined with 13C-PLFA profiling. *Soil Biology and Biochemistry*, *58*, 207–215. https://doi.org/10.1016/j.soilbio.2012.11.013

Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A., & Raizada, M. N. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*, *36*(4). https://doi.org/10.1007/s13593-016-0396-4

Tian, D., & Niu, S. (2015). A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters*, *10*(2). https://doi.org/10.1088/1748-9326/10/2/024019

Tomè, E., Tagliavini, M., & Scandellari, F. (2015). Recently fixed carbon allocation in strawberry plants and concurrent inorganic nitrogen uptake through arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, *179*, 83–89. https://doi.org/10.1016/j.jplph.2015.02.008

Townsend, A. R., & Howarth, R. W. (2010). Fixing the Global Nitrogen Problem. *Scientific American*, *302*(2), 64–71.

Turgeon, R. (1989). The sink-source transition in leaves. *Annual Review of Plant Physiology*, *40*(1), 119–138. https://doi.org/10.1146/annurev.biochem.64.1.721

von Rein, I., Gessler, A., Premke, K., Keitel, C., Ulrich, A., & Kayler, Z. E. (2016). Forest understory

plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. *Global Change Biology*, *22*(8), 2861–2874. https://doi.org/10.1111/gcb.13270

Wade, J., Maltais-Landry, G., Lucas, D. E., Bongiorno, G., Bowles, T. M., Calderón, F. J., … Margenot, A. J. (2020). Assessing the sensitivity and repeatability of permanganate oxidizable carbon as a soil health metric: An interlab comparison across soils. *Geoderma*, *366*(December 2019), 114235. https://doi.org/10.1016/j.geoderma.2020.114235

Walker, S., & Ogindo, H. O. (2003). The water budget of rainfed maize and bean intercrop. *Physics and Chemistry of the Earth*, *28*(20–27), 919–926. https://doi.org/10.1016/j.pce.2003.08.018

Wan, X., Huang, Z., He, Z., Yu, Z., Wang, M., Davis, M. R., & Yang, Y. (2015). Soil C:N ratio is the major determinant of soil microbial community structure in subtropical coniferous and broadleaf forest plantations. *Plant and Soil*, *387*(1–2), 103–116. https://doi.org/10.1007/s11104-014-2277-4

Wang, S. L., Nehring, R., & Mosheim, R. (2018). Agricultural Productivity Growth in the United States: 1948-2015. *USDA, Economic Research Service, Amber Waves Magazine*.

Wardle, D. A. (1998). Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. *Soil Biology and Biochemistry*, *30*(13), 1627–1637. https://doi.org/10.1016/S0038-0717(97)00201-0

Weiner, J., Campbell, L. G., Pino, J., & Echarte, L. (2009). The allometry of reproduction within plant populations. *Journal of Ecology*, Vol. 97, pp. 1220–1233. https://doi.org/10.1111/j.1365-2745.2009.01559.x

Xiao, Y., Li, L., & Zhang, F. (2004). Effect of root contact on interspecific competition and N transfer between wheat and fababean using direct and indirect 15N techniques. *Plant and Soil*, *262*(1–2), 45–54. https://doi.org/10.1023/B:PLSO.0000037019.34719.0d

Yang, T., Siddique, K. H. M., & Liu, K. (2020). Cropping systems in agriculture and their impact on soil health-A review. *Global Ecology and Conservation*, *23*, e01118. https://doi.org/10.1016/j.gecco.2020.e01118

Yuste, J. C., Peñuelas, J., Estiarte, M., Garcia-Mas, J., Mattana, S., Ogaya, R., … Sardans, J. (2011). Drought-resistant fungi control soil organic matter decomposition and its response to temperature. *Global Change Biology*, *17*(3), 1475–1486. https://doi.org/10.1111/j.1365-2486.2010.02300.x

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. *Biology and Fertility of Soils*, *29*(2), 111–129. https://doi.org/10.1007/s003740050533

Zhao, H., Li, X., Zhang, Z., Zhao, Y., Chen, P., & Zhu, Y. (2018). Drivers and assemblies of soil eukaryotic microbes among different soil habitat types in a semi-arid mountain in China. *PeerJ*, Vol. 2018. https://doi.org/10.7717/peerj.6042

Zosso, C. U., & Wiesenberg, G. L. B. (2021). Methylation procedures affect PLFA results more than selected extraction parameters. *Journal of Microbiological Methods*, *182*, 106164. https://doi.org/10.1016/j.mimet.2021.106164

Supplemental Information

Supplemental Table 3.1: Natural abundance (NA) isotope values for sampled tissue pools.

Tissue	Crop	Mean $\delta^{13}C$ (‰)	Std. error	Mean $\delta^{15}N$ (‰)	Std. error
Root	Barley	-23.3	0.82	-1.8	0.29
Root	Lentil	-22.4	1.08	-1.6	0.76
Root	Pea	-22.3	0.33	-1.8	0.22
Leaf	Lentil	-28.8	0.74	-2.3	0.51
Leaf	Pea	-28.4	0.81	-2.4	0.55

Supplemental Table 3.2: Raw average values of plant tissue $\delta^{13}C$ and $\delta^{15}N$ during the chase period.

Time after Label	Tissue	Pulse	Plant	Mean $\delta^{13}C$ (‰)	Std. error	Mean $\delta^{15}N$ (‰)	Std. error
1 day	Barley Root	Lentil	Barley	-25.5	0.979	-2.2	0.657
2 days	Barley Root	Lentil	Barley	-25.1	1.338	-4.4	0.690
6 days	Barley Root	Lentil	Barley	-23.8	0.838	-0.6	1.271
1 day	Barley Root	Pea	Barley	-25.7	0.656	-2.7	0.445
2 days	Barley Root	Pea	Barley	-25.6	1.013	-3.1	0.545
6 days	Barley Root	Pea	Barley	-23.3	0.907	-1.1	0.320
1 hour	Pulse Leaf	Lentil	Lentil	494.9	131.570	3.9	1.113
2 hours	Pulse Leaf	Lentil	Lentil	607.5	169.284	3.3	0.551
8 hours	Pulse Leaf	Lentil	Lentil	317.3	87.102	3.7	0.440
1 day	Pulse Leaf	Lentil	Lentil	435.9	139.231	4.6	1.213
2 days	Pulse Leaf	Lentil	Lentil	546.8	228.333	5.0	1.528
6 days	Pulse Leaf	Lentil	Lentil	579.7	197.286	9.5	4.055
14 days	Pulse Leaf	Lentil	Lentil	285.7	62.136	13.7	4.616
1 hour	Pulse Leaf	Pea	Pea	467.9	193.042	2.4	0.892
2 hours	Pulse Leaf	Pea	Pea	312.7	96.814	2.3	0.879
8 hours	Pulse Leaf	Pea	Pea	251.1	93.932	3.7	1.006
1 day	Pulse Leaf	Pea	Pea	456.2	226.968	7.3	1.351
2 days	Pulse Leaf	Pea	Pea	158.7	76.470	2.1	1.704
6 days	Pulse Leaf	Pea	Pea	218.7	99.626	5.4	1.544
14 days	Pulse Leaf	Pea	Pea	410.5	117.583	7.7	1.318
1 hour	Pulse Root	Lentil	Lentil	16.9	19.886	4.4	6.743
1 day	Pulse Root	Lentil	Lentil	-15.4	3.621	21.8	13.400
6 days	Pulse Root	Lentil	Lentil	-6.5	6.289	73.7	74.750
14 days	Pulse Root	Lentil	Lentil	-3.0	4.978	87.7	65.757
1 hour	Pulse Root	Pea	Pea	-10.1	4.953	6.6	9.899
1 day	Pulse Root	Pea	Pea	-18.4	1.133	-4.0	1.174
6 days	Pulse Root	Pea	Pea	-12.8	3.521	38.9	42.563
14 days	Pulse Root	Pea	Pea	-3.0	4.939	5.0	5.637

Supplemental Table 3.3: 2020 biomass yield averages (g/m^2) and standard errors for each cropping system. Data shown are for plots under full irrigation, where labeling occurred.

Treatment	Barley Biomass (g/m^2)	Std. error	Pulse Biomass (g/m^2)	Std. error	Total Biomass (g/m^2)	Std. error
Barley Monocrop	2387.5	206.53	0	*NA*	2387.5	206.53
Lentil Monocrop	0	*NA*	1504.2	126.55	1504.2	126.55
Pea Monocrop	0	*NA*	1622.2	70.09	1622.2	70.09
Barley/Lentil	1532.4	145.16	235.2	89.64	1777.6	63.84
Barley/Pea	1660.2	292.06	273.1	96.76	1933.3	227.98

Supplemental Table 3.4: PLFAs included in microbial groups

Microbial Group	PLFAs	Reference
General FAME	14:0, 16:0, 17:0, 18:0, 19:0	(von Rein et al., 2016)
Gram-Negative Bacteria	14:0 2OH, 14:0 3OH, 16:1ω9c, 17:1ω6c, 18:1ω7c, 18:1ω5c	(Frostegard & Baath, 1996; Zelles, 1999)
Eukaryotes	18:3ω6c, 20:5ω3c, 20:3ω6c, 22:6ω3c	(Zelles, 1999)
Fungi	18:2ω6c, 18:1ω9c	(Bååth, 2003; Frostegard & Baath, 1996)
Gram-Positive Bacteria	15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 anteiso, 17:0 iso	(Zelles, 1999)
Actinomycetes	16:0 10-methyl, 17:0 10-methyl	(Moore-Kucera & Dick, 2008)