Assessing Social, Ecological, and Nutritional Outcomes of Crop Diversification: Agroecological Transitions in Southern Brazil

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Natural Resources and Environment)
in the University of Michigan
2021

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Dedication

I dedicate this dissertation to the generations of strong women who came before me. To my grandmother and namesake, Anne Elizabeth: thank you for shaping my worldview with your infinite wisdom and playfulness. And to my mother Holly, the original agroecologist in my life: thank you for teaching me that to love Mother Earth is to love oneself.

Dedicação

Eu dedico esta dissertação para as gerações de grandes mulheres que vieram antes de mim. Para minha avó e homônimo, Anne Elizabeth: Obrigada por contribuir para a construção da minha percepção de mundo com sua sabedoria e alegria infinita. E para minha mãe Holly, a “original” agroecóloga da minha vida: obrigada por me ensinar que amar a mãe terra é amar a si próprio.

Acknowledgements

There are so many people to thank, which is something to be thankful for all on its own. I hope these words of thanks can begin to pay it forward to the helping hands and minds that have shaped this dissertation. First and foremost, I want to express my deep gratitude to the farming families and non-profit visionaries of Rede Ecovida, who are actively transforming their food systems and their lives to create a world where all people can access healthy food, "grown with love." Thank you especially to the 15 farming families who have shared their land and their lives with me during the last five years; each family has been a muse in its own way over the course of my PhD.

I owe many thanks to my dissertation committee. Thank you to my advisor, Jennifer Blesh—first, for inspiring me with her own community-engaged, interdisciplinary research, and then for constantly supporting me to pursue mine. We both knew that the interdisciplinary road wouldn't be easy, but Jennifer has provided unswerving encouragement and constructive feedback to help me see this dissertation through to a rigorous end. To Hannah Wittman, who has had a profound influence on my thinking and academic trajectory since I first set foot in Brazil in 2017. Thank you for helping me step back to see the bigger picture and for the regular reminders that work (and life) are better when they're fun. To Don Zak, who helped me see the magic and understand the science of soil ecology in the first year of my PhD. Thank you for giving me a foundational understanding of the natural world, and for getting me outside to experience environmental gradients first-hand through soil ecology field trips! Finally, thanks to Andy Jones, whose work showed me how to connect the dots between agroecosystems and human health and how to think critically about the causal pathways between them. Thank you for teaching me about food systems policy and for being generous with your time, especially in the more chaotic, formative stages of my research.

Of equal importance, I wish to thank my unofficial mentors. This dissertation could not have come to fruition without the support of my Brazilian advisors Jucinei José Comin and Ilyas Siddique at the Universidade Federal de Santa Catarina. Thank you to Jucinei, who opened his soils research lab and experimental farm to me and helped me overcome the many administrative and logistical hurdles that international fieldwork threw my way. Thank you to Cledimar Lourenzi and the many members of NEPEA soils lab who were so helpful in spite of our uneven positionality (especially Barbara Santos, Lucas Dupont, Monique Souza, and Matheus Junckes); I hope to repay the favor someday. Thank you to Marcelo Venturi and his team at the Ressacada Experimental Farm for productive soil management discussions and execution. Thanks to Ilyas, who not only taught me about structural equation modeling and statistics but also nurtured an incredible research group (with Fernando Joner) that was a constant source of friendship and ecological knowledge throughout the 12+ months I spent in Brazil. Thank you to my dear friends and fellow agroecologists Marinice Teleginski, Diego dos Santos, Hanna Schuler, Vicente Parra, and Alvaro Monteiro, for your insights and companionship. I give my sincere thanks also to my former mentors at Tufts University in Boston, including Selena Ahmed, Colin Orians, Alex Blanchette, and Laura Kuhl, each of whom guided and prepared me for an agroecology PhD.

Next, I must thank my wonderful family. Thank you to my parents, Holly and Clyde, who gave me an adventurous spirit, an open mind, and curiosity in the face of the unknown. Your constant flow of love and support have lifted me through all the tough times and reminded me to let my hair down to celebrate my successes. Mom, thank you for your empathy and cultural awareness, and for teaching me to dance, sing, garden, and take delight in the little things in life. These are all essential skills for getting a dissertation, it turns out! Dad, thanks for always being there when the logistics of life seem hard (you somehow manage to make it easy!), and thanks for passing down your common sense. I needed it for fieldwork. Thank you to my grandma Anne; she and my late grandpa Rod are probably the number one reason I ultimately chose to pursue a PhD. I am so grateful to them for instilling a love of books and learning in me from a young age. Thank you to the whole Johnson-Stratton-Schmitt Clan, including my brother Tim and sister-in-law Afton, aunt Molly and uncle Dan, mom's partner Chet, and cousins Josh, Jay, and Jess; your positive attitudes and honest interest in my work have always been a major motivation. Thank you to my life-long friends Tara Kola, Diane Adamson, Sarabeth Buckley, Minna Jacobson, Abby Keel, Hope Bretscher, and Johanna Mayer, who have provided much-needed perspective and sisterly love every step of the way. Last but certainly not least, thank you to the recent Dr. Tim G. Williams, my partner in crime, who adds a little brightness to every day, and whom I love dearly. Thank you for bringing levity, efficiency, and your can-do attitude into my life, and for the many dinners and lines of code you've engineered for us over the years. Your presence has made getting a PhD a whole lot sweeter. On a related note, I would also like to thank Tim's parents Ruth Blackman and Jon Williams for feeding and housing me for four months while I wrote this dissertation, and for encouraging me to get out and see the enchanting country of New Zealand.

Community has been foundational to this work. My communities of friends and colleagues have shifted over the last five years, from Michigan, to Brazil, and back again. In Michigan, the Blesh Lab has felt like home, and I am so thankful for the camaraderie, passion, and good-heartedness of that community. Thank you to Beth VanDusen, for her ample patience and mirth, for helping me accomplish more lab work than I ever thought possible, for her excellent organizational skills, and for making us wash and re-use plastic bags (it's better for the planet, and I secretly appreciated it). I am thankful for my peers Alison Bressler, Eliot Jackson, Etienne Herrick, and Vivian Valencia (and more recently Kent Connell), who have been there every step of the way, and who make Blesh Lab potlucks the envy of the town. Thanks to so many past and present Blesh Lab members with whom I have worked countless field and lab hours; I owe a particular debt of gratitude to Brianna Hansen, Dev Gordin, Carol Waldmann, Aminata Fofana, Sydney Fuller, and Thea Louis for steadfast and smart field and lab assistance. Other important communities in Michigan have included the Zak and Hunter labs, which have provided lab space, know-how, and wonderful, helpful people, including Peter Pellitier, Rima Upchurch, Leslie Decker, Amanda Meier, and Hillary Streit. Thanks again to Peter for his poetry. Thanks to Mark Hunter, who opened my eyes to the wonders of biogeochemistry and phytochemistry and helped me design my first crop nutrient experiments. And thank you to Drew Gronewold for making sure I know my stats. Another important community has been the MUSE (Michigan University-wide Sustainability and Environment) Initiative, through which I got to know countless like-minded folks, including my partner Tim Williams, as well as my adventure buddy and role model Beth Tellman and good friends and collaborators Nathalie Lambrecht, Michael

Lerner, Kyle Bushick, Zak Gersten, and James Arnott. I have also thoroughly enjoyed and benefited from the diverse scholars who make up the School for Environment and Sustainability (SEAS) PhD community, including Jon Sullivan, Katie Browne, Nicole Ryan, Dominic Bednar, Stefania Almazan Casali, Morteza Taiebat, Jake Hawes, Calli VanderWilde, Tara Easter, and many others.

In Brazil, I have also been blessed to meet and work with some incredible people. Top of the list are the caring and impassioned souls at CEPAGRO. Thank you to Letícia Filipini and Renata Lucas, who were my hardworking, laughing companions during my last field season in Brazil, and who will remain dear friends and colleagues into the future. Thanks to Charles Bagé Lamb for offering introductions to farmers, trips to the field, and boundless energy to propel the cover crops project forward and foster real change in rural Santa Catarina. Thanks to other members of the field and administrative teams in 2018 and 2019 for their efforts, including Rafael Beghini, Caru Dionisio, Clara Comandolli de Souza, Lorena Lucas, Nathalia Beck, Jefferson P. Mota, and Bruna Rentes. I would also like to thank the broader CEPAGRO community for providing such a welcoming home away from home: Erika Sagae, Eduardo "Dudu" Rocha, Gisa Garcia, and Maria Denis, among others. I am thankful as well for the time I got to spend in Brazil with members of the Wittman Lab, Dana James and Evan Bowness, especially in the early years. More recently, I feel intense gratitude for the friendships I was able to develop with intellectual and outdoorsy ladies in Brazil. Thanks especially to Jacira Prichula and her sister Janira, Priscila Prado, and Carlise Fuhr, both for helping me climb mountains and process mountains of cucumbers…

Thank you to ashtanga yoga. My yoga practice has been vital to my wellbeing while completing this PhD, and I am incredibly grateful to Angela Jamison of Ashtanga Yoga Ann Arbor and Ana Claudia (Kaká) of Samatva Yoga in Floripa for teaching me how to make space for mind-body growth in my twenties.

Finally, thank you to the sources of financial support that gave me so much freedom to pursue what I chose in my PhD. I received funding from many University of Michigan institutions, including the UM Library, the International Institute, the Department of Latin American and

Caribbean Studies, the School for Environment and Sustainability, the UM-Brazil Initiative and UM ADVANCE Program, the Botanical Gardens and Campus Farm, and Rackham Graduate School. Outside of the university, I am also grateful to have been awarded funding from the US National Science Foundation (NSF) Graduate Research Fellowship Program, NSF Graduate Research Opportunities Worldwide (GROW), NSF INTERN, the US Department of Education Foreign Language and Area Studies Program, the Brazilian Ministry of Education (CAPES), and the Conservation, Food and Health Foundation.

Agradecimentos

Há tantas pessoas a quem agradecer, que é algo para ser grato por si próprio. Espero que estas palavras de agradecimento sejam entregues às mãos e mentes que deram forma a esta tese. Em primeiro lugar, gostaria de expressar minha profunda gratidão para as famílias e voluntários visionários da Rede Ecovida, os quais estão transformando ativamente seus sistemas alimentares e vidas para criar um mundo onde pessoas possam acessar alimentos saudáveis, "cultivados com amor". Obrigada, especialmente, às 15 famílias agricultoras que têm compartilhado suas áreas e vidas comigo durante os últimos cinco anos, cada família tem sido um modelo à sua maneira ao longo do meu doutorado.

Eu devo muitos agradecimentos ao meu "comitê" de dissertação. Obrigado à minha orientadora, Jennifer Blesh - primeiro, por me inspirar com sua pesquisa interdisciplinar e, segunda, por me apoiar constantemente na minha busca. Nós duas sabíamos que o caminho interdisciplinar não seria fácil, mas Jennifer forneceu incentivo inabalável e comentários construtivos para me ajudar a levar esta tese a um final de qualidade. A Hannah Wittman, que teve uma influência profunda em meu pensamento e trajetória acadêmica desde que pus os pés no Brasil em 2017. Obrigado por me ajudar a enxergar melhor o todo e pelos lembretes regulares de que o trabalho (e a vida) são melhores quando divertidos. A Don Zak, que me ajudou a ver a magia e entender a ciência da ecologia do solo no primeiro ano do meu doutorado. Obrigado por me ajudar a compreender o mundo natural e me levar para experimentar os gradientes ambientais em primeira mão através das viagens de campo de Ecologia do Solo! Finalmente, agradeço a Andy Jones, cujo trabalho me mostrou como conectar os pontos entre os agroecossistemas e a saúde humana e como pensar criticamente sobre os caminhos causais entre eles. Obrigado por me ensinar sobre política de sistemas alimentares e por ser generoso com seu tempo, especialmente nos estágios mais caóticos e formativos de minha pesquisa.

De igual importância, desejo agradecer aos meus mentores não oficiais. Esta dissertação não poderia ter se concretizado sem o apoio de meus orientadores brasileiros Jucinei José Comin e Ilyas Siddique na Universidade Federal de Santa Catarina. Obrigado a Jucinei, que abriu seu laboratório de pesquisa de solos e fazenda experimental e me ajudou a superar os muitos obstáculos administrativos e logísticos que apareceram no caminho do trabalho de campo internacional. Obrigado a Cledimar Lourenzi e aos muitos membros do laboratório de solos do NEPEA que ajudaram muito (especialmente Bárbara Santos, Lucas Dupont, Monique Souza e Matheus Junckes); Espero retribuir o favor algum dia. Obrigado a Marcelo Venturi e sua equipe da Fazenda Experimental Ressacada pelas conversas e execução do manejo produtivo do solo. Agradeço a Ilyas, que não apenas me ensinou sobre modelagem de equações estruturais e estatística, mas também nutriu um incrível grupo de pesquisa (com Fernando Joner) que foi uma fonte constante de amizade e conhecimento ecológico durante os mais de 12 meses que passei no Brasil. Obrigado aos meus queridos amigos e companheiros agroecologistas Marinice Teleginski, Diego dos Santos, Hanna Schuler, Vicente Parra e Álvaro Monteiro, por suas opiniões e companheirismo. Agradeço sinceramente também aos meus ex-mentores na Tufts University em Boston, incluindo Selena Ahmed, Colin Orians, Alex Blanchette e Laura Kuhl, cada um dos quais me orientou e preparou para um PhD em agroecologia.

Em seguida, devo agradecer à minha família maravilhosa. Obrigado a meus pais, Holly e Clyde, que me incentivaram a ter um espírito aventureiro, uma mente aberta e curiosa diante do desconhecido. Seu fluxo constante de amor e apoio me ajudou em todos os momentos difíceis e me lembrou de comemorar meus sucessos. Mãe, obrigada por sua empatia e consciência cultural, e por me ensinar a dançar, cantar e ter prazer nas pequenas coisas da vida. Essas são todas habilidades essenciais para obter uma tese, ao que parece! Pai, obrigada por estar sempre presente quando a vida parece difícil (de alguma forma você consegue facilitar!), e obrigada por transmitir um pouco de bom senso. Eu precisava disso para o trabalho de campo. Obrigado a minha avó Anne; ela e meu falecido avô Rod são provavelmente a razão número um pela qual acabei escolhendo fazer um doutorado. Sou muito grato por eles incutirem em mim o amor pelos livros e pelo aprendizado desde jovem. Obrigado a todo o clã Johnson-Stratton-Schmitt, incluindo meu irmão Tim e cunhada Afton, tia Molly e tio Dan, companheiro da minha mãe Chet, e primos Josh, Jay e Jess; suas atitudes positivas e interesse no meu trabalho sempre foram uma grande motivação. Obrigado às minhas amigas de longa data, Tara Kola, Diane Adamson, Sarabeth Buckley, Abby Keel, Hope Bretscher, Minna Jacobson e Johanna Mayer, que forneceram a perspectiva necessária e o amor de irmã em cada passo no caminho. Por último, mas não menos importante, obrigado ao recente Dr. Tim G. Williams, meu parceiro em cada aventura, que adiciona um pouco de brilho a cada dia e a quem amo profundamente. Obrigado por trazer leveza, eficiência e positividade para a minha vida, e pelos muitos jantares e linhas de códigos que você projetou para nós ao longo dos anos. Sua presença tornou a obtenção de um PhD mais doce. Em uma nota relacionada, também gostaria de agradecer aos pais de Tim, Ruth Blackman e Jon Williams, por me alimentar e abrigar por quatro meses enquanto eu escrevia esta dissertação, e por me encorajar a sair e ver o país encantador da Nova Zelândia.

A comunidade foi fundamental para este trabalho. Meu grupo de amigos e colegas mudaram nos últimos cinco anos, de Michigan para o Brasil e vice-versa. Em Michigan, o Blesh Lab é um lar e sou muito grata pela camaradagem, paixão e bom coração desse grupo. Obrigado a Beth VanDusen, por sua paciência e alegria, por me ajudar a realizar mais trabalho de laboratório do que eu jamais pensei ser possível, por suas excelentes habilidades organizacionais e por nos fazer lavar e reutilizar sacolas plásticas (é melhor para o planeta, e eu secretamente apreciei). Agradeço meus colegas Alison Bressler, Eliot Jackson, Etienne Herrick e Vivian Valencia (e mais recentemente Kent Connell), que estiveram lá em cada momento e que fazem do Blesh Lab potlucks a inveja da cidade. Agradeço a tantos membros do Blesh Lab com quem trabalhei incontáveis horas de campo e laboratório; Tenho uma dívida particular de gratidão para com Brianna Hansen, Dev Gordin, Carol Waldmann, Aminata Fofana, Sydney Fuller e Thea Louis pela constante e inteligente assistência de campo e laboratório. Outros grupos importantes em Michigan incluíram os laboratórios de Zak e Hunter, que forneceram espaço de laboratório, conhecimento, pessoas maravilhosas e prestativas, incluindo Peter Pellitier, Rima Upchurch, Leslie Decker, Amanda Meier e Hillary Streit. Obrigado novamente a Peter por sua poesia. Agradeço a Mark Hunter, que abriu meus olhos para as maravilhas da biogeoquímica e fitoquímica e me ajudou a delinear meu primeiro experimento com qualidade nutritiva de culturas. Outra comunidade importante foi a Iniciativa MUSE (Meio Ambiente e Sustentabilidade para toda a Universidade de Michigan), por meio da qual conheci inúmeras pessoas que pensam como eu, incluindo meu companheiro Tim Williams, bem como minha companheira de aventura e modelo Beth Tellman e bons amigos e colaboradores Nathalie Lambrecht, Michael Lerner, Kyle Bushick, Zak Gersten e James Arnott. Eu também gostei muito e me beneficiei dos diversos estudantes que compõem a comunidade de PhD da "School for Environment and Sustainability" (SEAS), incluindo Jon Sullivan, Katie Browne, Nicole Ryan, Dominic Bednar, Stefania Almazan Casali, Morteza Taiebat, Jake Hawes, Calli VanderWilde, Tara Easter e muitos outros.

No Brasil, também tive a sorte de conhecer e trabalhar com pessoas incríveis. No topo da lista estão as almas carinhosas e apaixonadas do CEPAGRO. Obrigado a Letícia Filipini e Renata Lucas, que foram minhas companheiras trabalhadoras e sorridentes durante minha última temporada de campo no Brasil, e que continuarão sendo queridas amigas e colegas no futuro. Agradecemos a Charles (Bagé) Lamb por oferecer inúmeras apresentações aos agricultores, viagens ao campo, energia sem limites para impulsionar o projeto de culturas de cobertura e promover uma mudança real na zona rural de Santa Catarina. Obrigado a outros membros das equipes de campo e administrativas em 2018 e 2019 por seus esforços, incluindo Rafael Beghini, Caru Dionisio, Clara Comandolli de Souza, Lorena Lucas, Nathalia Beck, Jefferson P. Mota e Bruna Rentes. Também gostaria de agradecer à comunidade mais ampla do CEPAGRO por oferecer um lar tão acolhedor fora de casa: Erika Sagae, Eduardo "Dudu" Rocha, Gisa Garcia e Maria Denis. Agradeço também pelo tempo que passei no Brasil com os membros do Laboratório da Wittman, Dana James e Evan Bowness, especialmente nos primeiros anos. Mais recentemente, sinto intensa gratidão pelas amizades que pude desenvolver com mulheres intelectuais e que gostam de atividades ao ar livre no Brasil. Agradeço especialmente a Jacira Prichula e sua irmã Janira, Priscila Prado e Carlise Fuhr, por me ajudarem a escalar montanhas e processar montanhas de pepinos ...

Obrigado a ashtanga yoga. Minha prática de ioga foi vital para meu bem-estar ao concluir este doutorado, e sou extremamente grato a Angela Jamison do Ashtanga Yoga Ann Arbor e Ana Claudia (Kaká) do Samatva Yoga em Floripa por me ensinarem como abrir espaço para o crescimento mente-corpo em meus vinte anos.

Por fim, agradeço às fontes de apoio financeiro que me deram tanta liberdade para seguir o que escolhi no meu doutorado. Recebi financiamento de muitas instituições da “University of Michigan”, incluindo a “UM Library”, o “ International Institute”, o “Department of Latin American and Caribbean Studies”, a “School for Environment and Sustainability”, a “UM-Brasil Initiative” e o “UM ADVANCE Program”, o “Botanical Gardens and Campus Farm” e “Rackham Graduate School”. Fora da universidade, também sou grata por ter recebido financiamento do Programa de Bolsas de Estudo de Pós-Graduação da “ US National Science Foundation” (NSF), “NSF Graduate Research Opportunities Worldwide” (GROW), “NSF INTERN”, do “US Department of Education Foreign Language and Area Studies Program”, do Ministério da Educação (CAPES) e “Conservation, Food and Health Foundation”.

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Abstract

Global industrial agriculture drives worsening environmental and public health crises, prompting a search for transformative agricultural approaches that can maintain productivity while increasing social and environmental sustainability. One paradigm gaining traction is agroecology, a science, set of management practices, and social movement. Agroecological management applies ecological knowledge to manage crop diversity on farms (i.e., agroecosystems) and increase multiple functions, including soil nutrient cycling and crop productivity. However, due to the vast heterogeneity of farm management systems and environmental conditions, we lack a mechanistic understanding of transitions to agroecological management, or “agroecological transitions,” and their outcomes. This dissertation develops and applies an interdisciplinary approach to analyze the processes and outcomes of agroecological transitions on family farms in southern Brazil. Chapter 1 provides a conceptual framework for assessing social, ecological, and nutritional functions of agroecosystems across stages of the transition process and introduces the remaining chapters.

Chapter 2 focuses on social outcomes of agroecological transitions. I analyzed qualitative and quantitative management and socioeconomic data from 14 farms along an agroecological transition gradient to understand how changing management practices relate to farm income and working conditions. I found that agroecological farms (>5 years certified) achieved income parity and improved working conditions compared to conventional farms in the region. Farms in transition (0-5 years certified), however, struggled to manage ecological processes on their newly diversified farms, which increased work difficulty and reduced profits relative to both agroecological and conventional farms.

Chapter 3 examines the cascading relationships between farm management history, background soil fertility, crop diversification practices, and nitrogen cycling during agroecological transitions. I conducted a two-year experiment to test the performance of two legume-based diversification practices, cover cropping and intercropping, across the farm

gradient from Chapter 2. Structural equation modeling revealed that after accounting for variation in background fertility across sites, cover crop mixtures explained a further 67% of the variation in soil nitrogen availability at vegetable planting. Consequently, benefits of diversification practices for soil nitrogen cycling were ecologically relevant across farms within the short span of our experiment, with the greatest nitrogen availability overall on agroecological farms. Intercropped cucumber and snow pea had a yield advantage relative to monocrops across the farm gradient, contributing to a mean land equivalent ratio of 1.19 overall, and 1.27 in the second year of the experiment.

Chapter 4 evaluates how diversification practices affect two nutritional functions of vegetable agroecosystems. In a factorial field experiment, I studied the individual and combined effects of cover cropping and intercropping on cucumber and snow pea nutrient content and nutrient yield, including protein and six minerals. Total nutrient yield per area increased in the combined diversification treatment, driven by 5.3 times greater cucumber nutrient yield per plant compared to the control. The highest nutrient yield overall was in the cover cropped pea treatment, reflecting 11% higher protein yield per plant compared to the control. These findings provide initial evidence that diversified cropping systems can lead to agronomic biofortification of vegetable crops, particularly in low-input systems.

Chapter 5 synthesizes findings from the three studies and proposes an agenda for future research on crop diversification and agroecological transitions. This integrative dissertation illustrates that agroecological transitions, and the crop diversity they employ, offer a pathway toward agriculture that upholds farms' socioeconomic viability, bolsters key ecosystem functions including soil nutrient cycling, and produces more nutrient-rich crops.

Resumo

A agricultura industrial global impulsiona o agravamento das crises ambientais e de saúde pública, levando a uma busca por abordagens agrícolas transformadoras que podem manter a produtividade enquanto aumentam a sustentabilidade social e ambiental. Um paradigma que está ganhando força é a agroecologia, uma ciência, um conjunto de práticas de manejo e movimento que aplica o conhecimento ecológico para gerenciar diversos “agroecossistemas” agrícolas que suportam múltiplas funções, incluindo ciclagem de nutrientes, produtividade e controle de pragas. Devido à vasta heterogeneidade do manejo e das condições do solo, carecemos de uma compreensão mecanicista das transições para o manejo agroecológico, ou “transições agroecológicas”, e seus resultados. Esta tese desenvolve e aplica uma abordagem interdisciplinar para analisar os processos e resultados das transições agroecológicas na agricultura familiar no sul do Brasil. O Capítulo 1 fornece uma estrutura conceitual para avaliar as funções sociais, ecológicas e nutricionais dos agroecossistemas nas transições e apresenta os capítulos restantes.

O Capítulo 2 enfoca os resultados sociais das transições agroecológicas. Analisei o gerenciamento qualitativo e quantitativo e os dados socioeconômicos de 14 propriedades ao longo de um gradiente de transições agroecológicas para entender como as mudanças nas práticas de manejo se relacionam com a renda agrícola e as condições de trabalho. Descobri que as propriedades agroecológicas (>5 anos de certificação) alcançaram paridade de renda e melhoraram as condições de trabalho em comparação com as propriedades convencionais da região. As propriedades em transição (com certificação de 0 a 5 anos), no entanto, lutaram para gerenciar os processos ecológicos em suas propriedades recentemente diversificadas, o que aumentou a dificuldade de trabalho e reduziu os lucros em relação às propriedades agroecológicas e convencionais.

O Capítulo 3 examina as relações em cascata entre a história do manejo da área, a fertilidade do solo, as práticas de diversificação da cultura e as funções ecológicas relacionadas

ao ciclo do nitrogênio durante as transições agroecológicas. Eu conduzi um experimento de dois anos para testar o desempenho de duas práticas de diversificação baseadas em leguminosas, cultivo de cobertura e consórcio, em todo o gradiente das propriedades rurais do Capítulo 2. A modelagem de equações estruturais revelou que, além dos efeitos da fertilidade do solo de fundo, os consórcios de culturas de cobertura explicaram os mais de 67% da variação na disponibilidade de nitrogênio do solo no plantio de hortaliças. Consequentemente, os benefícios das práticas de diversificação para a ciclagem de nitrogênio do solo foram ecologicamente relevantes entre as propriedades rurais dentro do curto período de nosso experimento, com a maior disponibilidade geral de nitrogênio em propriedades agroecológicas. O pepino consorciado e a ervilha torta superaram a produção em relação às monoculturas em todo o gradiente das propriedades, contribuindo para uma rendimento relativo total (LER) de 1,19 no geral e 1,27 no segundo ano do experimento.

O Capítulo 4 avalia como as práticas de diversificação afetam duas funções nutricionais dos agroecossistemas vegetais. Em uma estação experimental no sul do Brasil, estudei os efeitos individuais e combinados do cultivo de cobertura e consórcio sobre o conteúdo e rendimento de nutrientes do pepino e ervilha torta, incluindo proteína e seis minerais. Descobri que o rendimento total de nutrientes por área aumentou no tratamento de diversificação combinada, impulsionado por 5,3 vezes maior rendimento de nutrientes do pepino por planta em comparação com o controle. O maior rendimento de nutrientes em geral foi no tratamento de ervilha cultivada com cobertura, refletindo um rendimento de proteína 11% maior por planta em comparação com o controle. Essas descobertas fornecem evidências iniciais de que sistemas de cultivo diversificados podem levar à biofortificação agronômica de hortaliças, particularmente em sistemas de baixo insumo.

O Capítulo 5 conclui com uma síntese das conclusões dos três capítulos e propõe uma agenda para pesquisas futuras sobre diversificação de culturas e transições agroecológicas. Esta dissertação integrativa ilustra que as transições agroecológicas e a diversidade de culturas que empregam oferecem um caminho para a agricultura que mantém a viabilidade socioeconômica das fazendas, reforça as funções essenciais do ecossistema como a ciclagem de nutrientes do solo e produz safras mais ricas em nutrientes.

Chapter 1 Introduction: Linking Social, Ecological, and Nutritional Functions of Agroecosystems Across Transitions

1.1 Agroecology: the science, practice, and movement in southern Brazil

Over the last century, the advent of global industrial agriculture has shifted the focus of food systems toward the production and consumption of a narrow range of staple crops (Pingali, 2015). The simplification and intensification of food systems have contributed to worsening environmental and public health crises (Matson et al., 1997; Cassidy et al., 2013; Swinburn et al., 2019). Researchers, governments, and international institutions now seek transformative agricultural approaches that can maintain productivity while increasing the social and environmental sustainability of food systems (IAASTD, 2009; HLPE, 2019). One paradigm gaining momentum in the global arena is agroecology, which encompasses a science, a set of practices, and a grassroots movement at the intersection of social and ecological systems (Wezel et al., 2009).

As a scientific discipline, agroecology applies ecological theory and knowledge to improve understanding and management of agricultural systems, as well as the broader food system (Gliessman, 2014). In agroecology, a farm is conceptualized as an “agroecosystem,” which is subject to the same ecological principles as a natural ecosystem but with higher levels of human influence through active management. As a set of practices, agroecology minimizes chemical inputs such as fertilizers and pesticides and replaces them with a greater diversity of crop and livestock species, each of which serves a specific ecological purpose, or “function,” in the agroecosystem (Altieri, 1999). The greater the number of species with traits that contribute to different ecological functions, the greater the “functional diversity” of the agroecosystem. Functional diversity is often associated with higher overall levels of desired agroecosystem functions, including soil nutrient cycling and retention, soil carbon (C) accrual, pollination, pest control, and crop productivity (Cadotte et al., 2011; Wood et al., 2015; King and Blesh, 2018).

Agroecological management intentionally increases crop functional diversity in space and time through practices such as cover cropping, intercropping, agroforestry, and crop-livestock integration (Kremen et al., 2012; Wezel et al., 2014). Finally, as a movement, agroecology has roots as an "alternative agriculture" in protest of industrial models of food production that became normalized during the Green Revolution in the 1970s (Wezel et al., 2009). Social movements for agroecology argue that by increasing crop diversity, reducing reliance on purchased inputs, and directly connecting producers and consumers, agroecological management also bolsters autonomy, equity, and sustainable development in rural communities (da Costa et al., 2017; Mier et al., 2018).

Southern Brazil, which consists of the states of Santa Catarina, Rio Grande do Sul, and Paraná, is an oft-cited example of the successful confluence of agroecology in science, in practice, and in social movements (Wezel et al., 2009; Mier et al., 2018). In southern Brazil, the movements and practices of agroecology came first. A foundational group was the farmer network Rede Ecovida de Agroecologia (Ecovida Network of Agroecology, henceforth "Rede Ecovida"), which traces back to the 1970s but was formally founded in Rio Grande do Sul in 1998 (Mier et al., 2018). Also in the 1990s, the land redistribution movement Movimento dos Trabalhadores sem Terra (MST) added agroecology to its platform, alongside its main objective of agrarian reform. As the popularity of these social movements grew, so did a robust, interdisciplinary scientific community studying agroecology in the region (da Costa et al., 2017). The strong presence of agroecology across these three domains drew me to choose Santa Catarina as the site of my dissertation research.

Taking a social-ecological approach, my dissertation engages with agroecology directly as a science and a set of farm management practices. The context of my research, however, is tightly interwoven with the agroecology movement Rede Ecovida. My main partner organization in this work was a non-profit member organization of Rede Ecovida, called the Center for the Study and Promotion of Group Agriculture (CEPAGRO), and through this connection I recruited conventional, transitioning, and established agroecological farmers to participate in the research that constitutes Chapters 2 and 3 of this dissertation. All farms in the study were in Rede Ecovida's sphere of influence, but they had differential uptake of agroecological practices. The agroecology movement’s influence in the region made it possible to take a novel approach to studying the effects of agroecological practices on farms – I collected data across conventional, transitioning, and agroecological farms, which together formed an “agroecological transition gradient.” Along this gradient, this dissertation assesses intersecting social, ecological, and nutritional functions of agroecosystems, as I describe below.

1.2 Social, ecological, and nutritional functions of agroecosystems[1]

Social functions of agroecosystems

Agriculture is a fundamentally social endeavor. It brings together groups of individuals to work toward the common goal of food production and the maintenance of human lives through its consumption. In today’s world, this purveyance of food for human sustenance occurs in large part through markets, though 51-77% of the world’s food continues to be grown by small and medium-scale farmers (≤50 ha) who commonly eat at least part of what they produce directly (Herrero et al., 2017). Drawing from comprehensive indicator frameworks (Cabell and Oelofse, 2012; Tittonell, 2020), we can identify key social functions of agroecosystems. These include social self-organization through agricultural and consumer collectives, reflection and shared learning through farmer networks, and building human capacities with knowledge and skills gained through agricultural experience (Cabell and Oelofse, 2012). The predominance of markets for the sale and distribution of crops means that the social functions of agroecosystems also encapsulate the economic systems required to maintain farming livelihoods. Thus, additional social functions include financial independence and autonomy in the face of volatile global markets, as well as interdependence through access to shared local markets (Dumont et al., 2016b; Tittonell, 2020). Socioeconomic functions of agroecosystems, including farm income, working conditions, and market access, are the focus of Chapter 2 of this dissertation.

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[1] The conceptual framework in this chapter was adapted from a published paper written collaboratively with co-authors Laura Kuhl and Jennifer Blesh (Stratton et al., 2020).

Ecological functions of agroecosystems

Basic ecosystem processes, including fluxes of energy and nutrients and interactions among species, drive different functions in agroecosystems. Some examples of functions that are central to nutrient cycling in agroecosystems include primary production, decomposition, and biological nitrogen (N) fixation by legume species and their microbial symbionts. Ecological processes are driven in part by abiotic and biotic conditions outside of farmers' control, but farmers are able to alter many agroecosystem functions through management practices, whether intentionally or unintentionally (Drinkwater et al., 2008). Past work suggests that intentional management of ecological processes through agroecological practices (Kremen and Miles, 2012; Wezel et al., 2014) results in resilience of desirable, or productive, states in agricultural systems (Shennan, 2008; Peterson et al., 2018). Such strategies can promote long-term agroecosystem functioning and stability (Bailey and Buck, 2016).

Agroecological management practices, in particular, can improve nutrient uptake in cropping systems by augmenting biotic interactions to enhance nutrient cycling (Brooker et al., 2016). For example, increasing the diversity of crop rotations with cover crops is a practice that can improve multiple ecosystem functions at once, also called agroecosystem "multifunctionality" (Snapp et al., 2005; Finney and Kaye, 2016; Blesh, 2018). Among other functions, cover crops in the legume family supply N and carbon to soils through biological N fixation and photosynthesis. These N and C inputs add to pools of bioavailable soil nutrients, as cover crops are generally not harvested but instead are incorporated into the soil at the end of the season as "green manures." This agroecological practice has therefore been shown to increase internal nutrient cycling and nutrient availability to primary crops, with potential to increase productivity over time (Wander et al., 1994; Blesh, 2019). More broadly, the addition of legume cover crops to crop rotations introduces additional plant traits that influence ecosystem functions, contributing to both agricultural biodiversity and crop functional diversity (Wood et al., 2015). Chapter 3 of this dissertation quantifies the effects of two crop diversification practices, cover cropping and intercropping, on several interacting ecological functions in agroecosystems: legume N fixation, soil N cycling and retention, and crop productivity.

Nutritional functions of agroecosystems

As an extension of ecosystem functioning, DeClerck and colleagues (2011) proposed that, given agroecosystems' primary goal of food production for human nutrition and health, nutritional functions of agroecosystems should be measured alongside their social and ecological counterparts. Although their study proposed one nutritional function, nutritional functional diversity, multiple nutritional functions have rarely been considered in assessments of agroecosystem performance, nor have nutritional functions been explicitly related to underlying ecological functions. In this dissertation, I conceptualize nutritional functions at the intersection of social and ecological functions of agroecosystems, because nutrients in edible crops and livestock are the biogeochemical bridge between agroecosystems and human nutrition.

Other nutritional functions of agroecosystems include the quantity, diversity, and nutritional composition and content of crops produced (Remans et al., 2011; Allen et al., 2014). Importantly, these indicators of nutritional function consider more than just yield or productivity, which has been the dominant metric for assessing agroecosystem performance since the Green Revolution (Cassidy et al., 2013). Favoring productivity as the sole goal of agroecosystems can falsely place household food security and rural livelihoods at odds with critical ecological functions (Zhang et al., 2007; Bennett et al., 2009; Nelson et al., 2009). Just as farm management practices impact ecological functioning, they also affect nutritional functions, such as the nutritional quality of crops, as well as their productivity. Ecological functions therefore affect the overall ability of an agroecosystem to provide nutritional functions to people—through the production of a diverse selection of nutritious foods. Extending beyond the agroecosystem level, recent high-profile reports have highlighted agroecological transitions as an innovative approach to enhance food security and nutrition globally (IAASTD, 2009; HLPE, 2019).

Agroecological management frequently results in the nutritional diversification of cropping systems. Diversified farms have high levels of interaction between plant species, and between plants and microorganisms, which can maximize the efficiency of nutrient use on farms (e.g., Matson et al. 1997, Shennan 2008, Kremen and Miles 2012). When nutrient use efficiency (defined as total nutrient harvested/total nutrient input) of crops increases, there is greater uptake of nutrients by crop species, which can increase crop nutrient yields. Greater nutrient use efficiency also tends to correspond with reduced nutrient losses through runoff, leaching, or other pathways (Robertson and Vitousek, 2009). Such management practices thus have direct impacts on environmental sustainability as well as the quantity and nutritional composition of food produced and consumed in an agroecosystem. At the same time, it is important to acknowledge potential tradeoffs between management strategies that maximize ecological or nutritional functions (e.g., Kremen and Miles 2012, Power 2010), often by favoring short-term nutritional functions (e.g., crop yield or income from crop sales in a single season) over longer-term ecological ones (e.g., soil organic matter formation, C storage, and nutrient retention) (e.g., Steffan-Dewenter et al. 2007).

Continuing the example of farm diversification with cover crops, we can identify specific links between the ecological and nutritional functions derived from this practice. Nutritional functions include supporting crop yields with nutrient inputs from legume N fixation (Drinkwater et al., 1998), and increasing availability of other nutrients that can make crops more nutrient-rich. Soil phosphorus (P), for example, can be solubilized by acidic and enzymatic root exudates from legumes in species mixtures (Hinsinger et al., 2011; Xue et al., 2016). Reduced soil erosion is also likely to improve crop yields and nutrient availability, especially if the system in question is a low-input farm on steep terrain (Vanek and Drinkwater, 2013). Increased yields in a resource-poor agricultural context could correspond to improved household food security or self-sufficiency, or to increased incomes, if crops are sold (Sibhatu et al., 2015). Shifts in both ecological and nutritional functions of agroecosystems in this example illustrate the interactions resulting from farmer management decisions that can influence agroecosystem functions.

Given the complexity of food systems, nutritional functions of agroecosystems are only one step in the process of achieving human nutrient adequacy. A standard set of indicators for nutrition is the Food and Agriculture Organization's (FAO) four dimensions of food security: availability, access, utilization, and stability (FAO, 2008). Agroecosystems relate most directly to food availability, access, and stability, though specific agriculture-nutrition linkages are highly context-dependent. As defined in the 1996 World Food Summit, food security is "physical and economic access to sufficient safe and nutritious food that meets [one's] dietary needs and food preferences for an active and healthy life" (FAO, 2008). The idea that adequate nutrition, and not only sufficient caloric intake, is required for long-term health is an important aspect of the FAO's definition and therefore critical to nutrient provisioning at the agroecosystem level (Jones et al., 2016). To fully assess agroecosystem functions, then, social, ecological, and nutritional indicators should be integrated into a single framework.

Indicators linking social, ecological, and nutritional functions of agroecosystems

In Table 1.1, I summarize indicators of social, ecological, and nutritional agroecosystem functions relevant to this dissertation. While similar indicators could be used at larger spatial scales, such as landscapes or regions, in the chapters that follow, I apply them at the agroecosystem level. Though they do not directly measure outcomes related to human nutritional status, the listed "nutritional" indicators relate conceptually to the FAO's dimensions of food security (FAO, 2008). A more comprehensive discussion of interrelated ecological and nutritional functions of agroecosystems is published elsewhere (Stratton et al., 2020).

Table 1.1. Summary table of indicators used to represent social, ecological, and nutritional functions of agroecosystems in this dissertation.

Relevant Dissertation Chapter(s)	Indicator Type	Indicator	Agroecosystem Function
2	Social	Financial independence	Secure long-term economic viability and autonomy of the farming operation, including net income and access to markets
2	Social	Enabling working conditions	Support farm household physical, mental, and emotional wellbeing with reasonable working hours, occupational health, and job satisfaction
2, 3, 4	Ecological	Crop and livestock diversity	Fill distinct ecological niches and contribute to long-term productivity by varying and integrating crop and livestock species over time and in space
3, 4	Ecological	Total crop yield per area	Produce crops over time and under variable soil and climate conditions
3, 4	Ecological	Beneficial species interactions	Facilitate crops' nutrient uptake, growth, and reproduction through beneficial interactions

			within and between trophic levels (e.g., pollinators, soil biota)
3, 4	Ecological	Functional diversity and redundancy	Enable a functional safety net by planting crops with diverse ecological functional traits (e.g., biological N fixation and N retention) and associated non-crop species diversity
4	Nutritional	Total nutrient yield per area	Balance crop yield and nutrient content to produce nutrient-rich crops for human consumption
4	Nutritional	Edible crop quality	Increase crop nutrient content and elicit phytochemical responses through facilitative species interactions, improving crop nutritional quality for human diets
4, 5	Nutritional	Nutritional functional diversity	Fulfill nutritional needs for household diets by growing crop species that provide complementary and diverse nutrients
5	Nutritional	Access to a diversified diet	Provide access to diverse food crops, potentially impacting diet quality

1.3 Transitions to agroecological management, or “agroecological transitions”

The concept of agroecological transitions originates in the sustainability transition, a critical area of inquiry that examines social-ecological drivers and outcomes of systems change toward sustainability (Hinrichs, 2014; Ollivier et al., 2018). The agroecological transition is marked by a switch from farming practices reliant on external chemical inputs and low crop diversity to one that manages species diversity to support a broad range of biotic interactions that contribute to agroecosystem functions (Tomich et al., 2011; Blesh and Wolf, 2014). Agroecological transitions have become a pressing topic of concern, as dominant forms of agriculture are contaminating water sources, degrading soil resources, and reducing species diversity at a rapid pace and on a global scale (Matson et al., 1997; Garibaldi et al., 2017). But shifting the structure of agricultural systems from industrial (conventional) to agroecological is not only a biophysical process; it is one directly tied to farmer livelihoods and decision-making, which take place in the context of the globalized, production-oriented food system strongly influenced by the Green Revolution (Bezner-Kerr, 2012).

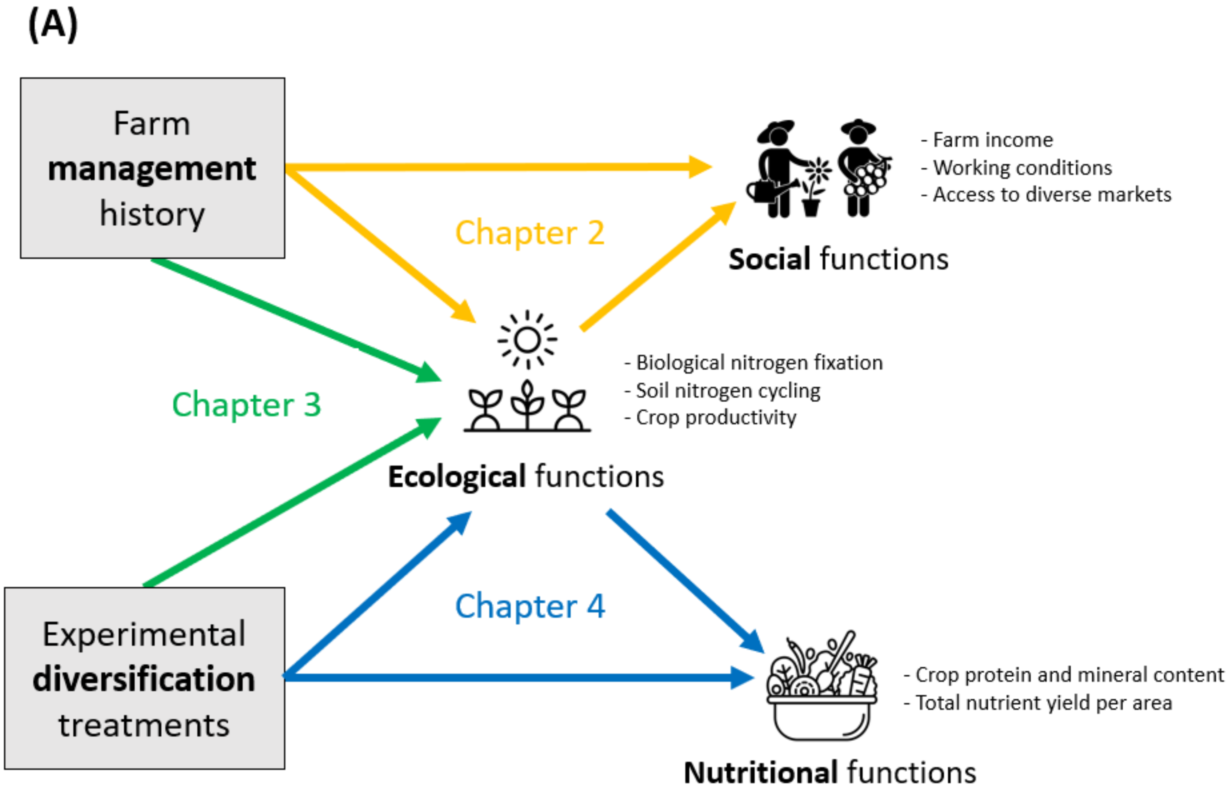
From a management perspective, farms undergoing agroecological transitions gradually increase their crop and livestock diversity, soil cover (e.g., with perennials and cover crops), and use of ecological nutrient and pest management strategies (see Chapter 2). Diversification of crop and livestock species in the agroecosystem is a fundamental structural change involved in agroecological transitions (Gliessman, 2014). Increasing agricultural species diversity can shift the ecosystem state from one emphasizing the crop production function to one that contributes to multiple ecosystem functions, particularly when species have complementary functional traits in space and time (Kremen et al., 2012; Wood et al., 2015; Tittonell, 2020). Planting multiple, interacting crop species in a field through intercropping, for example, increases spatial functional diversity and can augment nutrient retention and productivity in the agroecosystem (Brooker et al., 2016). Continuous soil cover can build soil organic C and retain N and P in agroecosystems by maximizing the extent and functional complementarity of living crop biomass (Isbell et al., 2017; King and Blesh, 2018). Non-harvested cover crops increase the temporal functional diversity of a cropping system when planted in the place of bare fallows. Complementing the use of cover crops and perennials for soil cover, an ecological approach to nutrient supply is to couple C, N, and P inputs through organic nutrient sources, including biological N-fixation by legumes and composted food scraps, yard clippings, and manure. By coupling nutrient inputs, this approach pairs decomposition and primary production processes (Drinkwater and Snapp, 2007b). Ecological pest management employs intentional arrangements of crop and livestock species and other methods of biological control to modulate disease and pest populations (Letourneau et al., 2011; Kremen and Miles, 2012). Farmers can leverage crop functional diversity to accomplish each of these goals over the course of agroecological transitions. My dissertation studies the intersections between agroecosystem diversification, soil cover, and ecological nutrient management by manipulating crop functional diversity in space and time.

We have seen that agroecological practices such as intercropping and cover cropping with diverse species can support agricultural production and other ecosystem functions during transitions (Bedoussac et al., 2015; Finney and Kaye, 2016). Yet the promise of functionally diversified cropping systems is not consistently realized on working farms. Farms with differing soil and climate conditions, as well as prior management regimes, can experience uneven results when implementing diversification practices (Reiss and Drinkwater, 2020). The increased complexity of managing diverse crop and livestock species can be especially challenging for farmers in the early years of agroecological transitions (Martini et al., 2004). Thus, to assess the processes and outcomes of agroecological transitions, it is essential to measure the performance of management practices across a range of farm conditions. There is a dearth of prior research attempting to understand this variability as farms transition to agroecological management, limiting our ability to predict how management practices will perform at different stages of transition. By studying the effects of two diversification practices, cover cropping and intercropping, on agroecosystem functions across a farm transition gradient, this dissertation fills this need.

1.4 Assessing agroecosystem functions across transitions: a conceptual framework

Encompassing the suite of management decisions farmers make to shift their production from simplified traditional (low-input) or conventional (high-input) approaches toward agroecological management (Tittonell, 2020), transitions are mediated by intertwined social and ecological factors. Factors especially important for agroecological transitions include social networks, access to resources, ecological knowledge, and environmental conditions (Ollivier et al., 2018). Understanding transition outcomes therefore requires a social-ecological perspective, and recent work has proposed that integrated social-ecological experiments provide an innovative approach to studying these real-world patterns (Gaba and Bretagnolle, 2020).

Agroecosystems can be designed to provision not only ecological functions, such as soil nutrient cycling and retention, but also social and nutritional functions, including farm income and nutrient-rich foods. Understanding the interactions between social, ecological, and nutritional functions of agroecosystems across agroecological transitions is the overarching goal of this dissertation (Figure 1.1). In Chapter 2, I assess the relationship between ecological and social functions of agroecosystems by studying farm income, working conditions, and market access across three stages of agroecological transition: conventional (not certified), transitioning (0-5 years certified agroecological through Rede Ecovida), and agroecological (>5 years certified). In Chapter 3, I test the individual and combined effects of two diversification practices, grass-legume cover cropping and legume-cucurbit intercropping, on ecosystem functions across the farm gradient from Chapter 2. Specifically, I measure the cascading effects of farm management history, soil fertility, and crop diversification on the ecological functions of biological N fixation, soil N cycling, and crop productivity. Finally, in Chapter 4 I evaluate the effects of the same diversification practices on nutritional functions of agroecosystems, measuring nutrient content (protein and six minerals) and nutrient yield of two vegetable crops in a controlled field experiment. Detailed summaries of each chapter are included below.



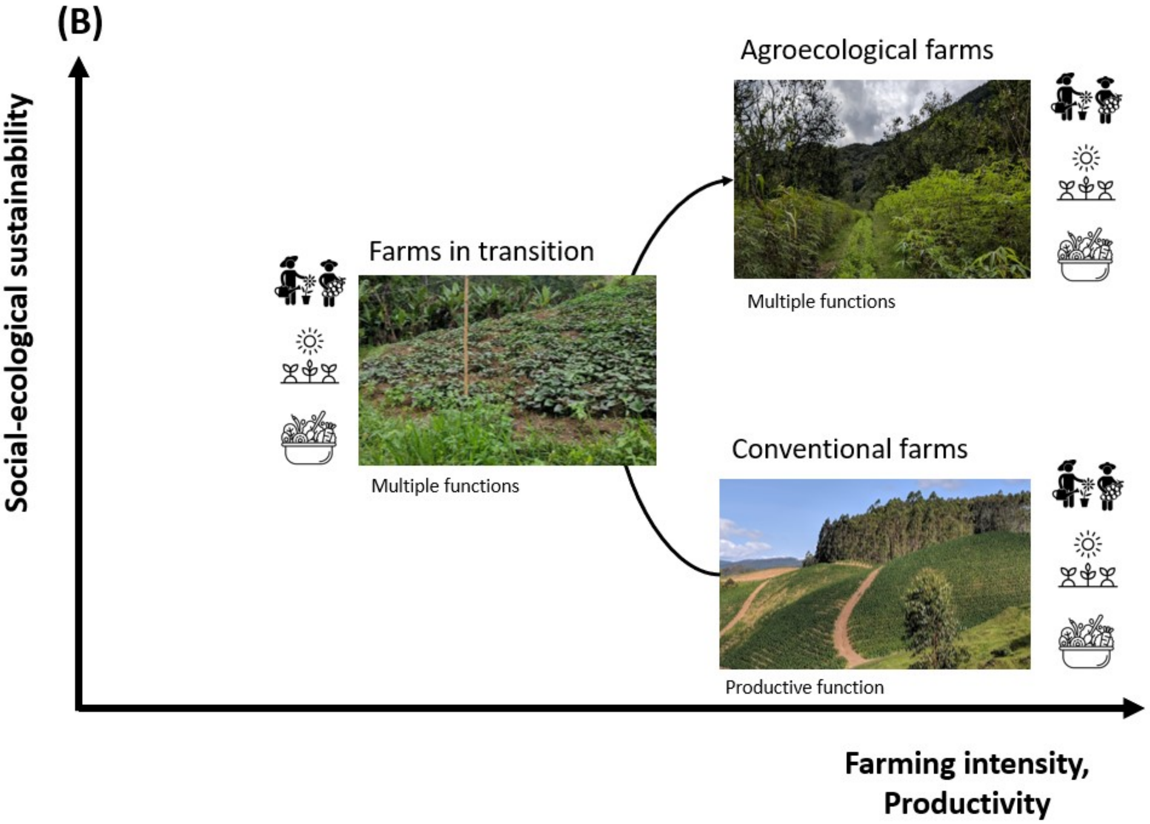


Figure 1.1. Conceptual framework for the dissertation. **(A)** Summary of dissertation chapters and the interactions between social, ecological, and nutritional functions of agroecosystems that I assessed in three interconnected studies. **(B)** Dissertation research design. Social, ecological, and nutritional functions of agroecosystems are measured across a transition gradient. Agroecological transitions represent shifts from agricultural systems that focus on yields of a small number of commodity crops to those that simultaneously provide multiple social and ecological functions. Farms in transition are in the process of restoring multiple functions (e.g., soil fertility) and thus have yet to achieve the levels of productivity and sustainability that characterize agroecological farms.

1.5 Summary of dissertation chapters

Chapter 2: Diversification supports farm income and improved working conditions during agroecological transitions in southern Brazil. Managing crop diversity to improve agroecosystem functioning can provide economic co-benefits to farmers through price premiums for organic products, reduced input costs, and other mechanisms (Bowman and Zilberman, 2013; Valencia et al., 2019), but the socioeconomic outcomes of agroecological transitions have never been studied. In this chapter, I sought to characterize how farm management practices and socioeconomic conditions differ across a purposively selected gradient of farms transitioning from conventional tobacco to diversified agroecological production. To do so, I sampled 14 farms along a transition gradient and conducted crop diversity and management surveys and semi-structured, in-depth interviews with household members. Using these data, I assessed indicators of ecological management, financial independence, and working conditions across three transition stages – conventional, transitioning, and agroecological. I aimed to study agroecological transitions in a region where farmers have access to knowledge, resources, and diverse markets to support their transitions, motivating an extreme case sampling approach (Patton, 2014). In-depth interviews across a gradient of transitioning farms enabled my understanding of how different stages of transition influence socioeconomic outcomes, as well as ecological ones, based on farmers' experiences. When analyzed through the lens of qualitative causal explanation (Maxwell, 2004, 2012), case studies can provide valuable insight into causal processes and mechanisms of change in a specific social-ecological context (Magliocca et al., 2018). For this case study in southern Brazil, I asked the following **research questions:**

1. To what extent do farms' management practices at different stages of transition align with specific ecological indicators?
2. How does transition stage influence income and working conditions on farms undergoing agroecological transitions?

Chapter 3: Assessing cover crop and intercrop performance along an agroecological transition gradient. The impacts of legume-based crop diversification on ecosystem functions (e.g., soil N cycling and productivity) can vary across heterogeneous farm soil conditions and management histories (Barel et al., 2018; Blesh, 2019). This variability may negatively impact farms undergoing agroecological transitions, particularly if they encounter inconsistent outcomes of practices such as cover cropping and intercropping that aim to improve agroecosystem performance. Prior on-farm research has identified positive effects of intercropping, crop rotations with semi-perennials (Snapp et al., 2010; Mwila et al., 2021), and annual cover cropping (Vanek and Drinkwater, 2013; Vanek et al., 2020) on crop productivity and other ecosystem functions in low-fertility regions. However, the integrated effects of cover cropping and intercropping on soil N cycling and its relationship to productivity in nutrient-limited conditions remain to be tested. To address this research gap, in Chapter 3 I report on results from a two-year experiment that assessed the ecological processes associated with two practices designed to increase crop temporal and spatial functional diversity relative to controls: grass-legume cover cropping and cucurbit-legume intercropping. The field experiment spanned 14 farms in southern Brazil with different long-term management histories representing three stages of agroecological transition: conventional (not certified), transitioning (0-5 years certified agroecological), and agroecological (>5 years certified). Using structural equation modeling, I subsequently analyzed the strength of relationships between measured pre-experimental management and soil characteristics, cover crop performance (biological N fixation, aboveground biomass N production), soil N cycling (potentially mineralizable N, inorganic N availability), and intercrop performance (land equivalent ratio for yield and N yield).

Chapter 3 Research Questions:

1. How does management history influence soil fertility on farms at different stages of agroecological transition?
2. How does soil fertility status affect cover crop N assimilation and N availability for subsequent crops?
3. How does soil fertility status influence vegetable yields in intercrop relative to monocrop?

Chapter 3 Hypotheses:

1. Agroecological management history will lead to higher soil fertility among farms in the study.
2. N supply from fixation and subsequent decomposition of the cover crop mixture will increase soil N availability compared to the fallow across all farms, with highest cover crop biomass production and N availability on agroecological farms.
3. Lower-fertility soils and lower N inputs from the cover crop will lead to higher yields from vegetable intercrops relative to monocrops, due to complementary nutrient uptake in both species and greater facilitation from legume N fixation.

Chapter 4: Cover cropping and intercropping increase total nutrient content and nutrient yield in vegetable agroecosystems. Diversified vegetable production has potential to improve agricultural sustainability and provide nutritious foods for healthy diets (Schreinemachers et al., 2018; Stratton et al., 2021a), but little research has explored how practices to diversify vegetable agroecosystems could affect the nutrient content of crops. The few studies that have tested crop nutrient content following diversification interventions found that incorporating legume cover crops into rotations can increase both N and micronutrient (e.g., Zn) uptake in subsequent grain crops (Turmel et al., 2009; Watson et al., 2012). Similarly, functionally diverse intercrops, such as beans and squash, tend to have complementary resource use and facilitative interactions known to increase nutrient uptake and concentrations in edible crops (Zuo and Zhang, 2009; Brooker et al., 2016; Xue et al., 2016). In a factorial experiment in southern Brazil, I tested the effects of two practices that increase crop functional diversity in space and time, grass-legume cover cropping and cucurbit-legume intercropping. I then measured the nutrient content (sum of protein and six minerals per 100g fresh vegetable) and nutrient yield (nutrient content multiplied by crop yield) of vegetables in each treatment. Vegetable species were selected for their distinct ecological and nutritional functional traits: snow pea is a climbing, N-fixing legume high in protein, and cucumber is a groundcover cucurbit high in minerals such as potassium (K).

Chapter 4 Research Questions:

1. What are the individual and combined effects of cover cropping and intercropping on crop nutrient content and nutrient yield?
2. How do the effects of these diversification practices on crop nutritional quality vary in species with distinct nutrient acquisition strategies?

Chapter 4 Hypotheses:

1a. The addition of a functionally diverse cover crop mixture will increase the supply, retention, and availability of nutrients for the subsequent vegetable crops relative to fallows, thereby increasing crop yields and nutritional quality.
1b. Intercropping cucumber and snow pea will increase vegetable nutrient yields and nutrient content relative to monocropped controls, due to niche partitioning and direct facilitation between species through biological N fixation and solubilization of micronutrients.
1c. There will be an additive effect of increasing spatial (intercrop) and temporal (cover crop) diversity on vegetable nutrient yields and nutrient content.
2a. Cover cropping will increase cucumber N uptake and yields to a greater extent than for pea.
2b. Effects of intercropping will be similar between crop species.

2c. In combined diversification treatments, cucumber will benefit more strongly from the increased availability of N and minerals from cover crop residues and from the legume intercrop, while snow pea will be less competitive in a high-N soil environment following cover cropping.

Chapter 5: Concluding thoughts and future research proposals. In the final chapter, I discuss the main contributions of this dissertation to the field of agroecology, as well as potential implications for food systems and sustainable development more broadly. I highlight novelties, acknowledge shortcomings, and propose new research directions that can expand on the methods, spatial and temporal scales, and policy applications of this work.

Chapter 2 Diversification Supports Farm Income and Improved Working Conditions During Agroecological Transitions in Southern Brazil

Abstract

Management of crop diversity for improved agroecosystem functioning can provide economic co-benefits to farmers. Yet, there remain critical gaps in understanding how farm management practices evolve through agroecological transitions, and how agroecological practices affect socioeconomic outcomes such as income and working conditions. We conducted a case study of farms transitioning from conventional tobacco production to diversified agroecological management in a participatory certification network in southern Brazil. We purposively sampled farms along a transition gradient and conducted crop diversity and management surveys and semi-structured, in-depth interviews with household members. Using these data, we assessed indicators of ecological management, income, and working conditions across three transition stages – conventional, transitioning, and agroecological. We found that ecological management indicators increased in magnitude and evenness by transition stage, as transitioning farmers shifted toward practices that support ecological complexity. Agroecological farmers utilized system redesign, a transformative approach to agroecosystem management, rather than efficiency-based or substitution-oriented practices adopted by conventional and transitioning farmers. While farms in transition reported more difficult working conditions and lower incomes, agroecological farmers had similar per capita working hours and improved work quality and occupational safety relative to conventional farmers in the region. On a per capita basis, experienced agroecological farmers earned similar net agricultural incomes and higher net household incomes than conventional farmers, accomplished by reducing agricultural expenses and diversifying their markets and livelihoods. Our study is the first to our knowledge to use a transition gradient approach to examine how agroecological transition stage affects both ecological and socioeconomic indicators on farms, providing insights into the processes and pathways by which farmers overcome challenges during transitions. Results highlight the

potential for stable profits and improved working conditions on farms following agroecological transitions, within a supportive policy and market context.

2.1 Introduction[2]

The United Nations Sustainable Development Goals demonstrate rising global acknowledgement that in order to feed a growing population through 2030 and beyond, agriculture must become more sustainable and equitable (Blesh et al., 2019). Agricultural shifts toward more biodiverse and biologically-mediated models of food production are called "agroecological transitions" (Ollivier et al., 2018). While the phenomenon is well-recognized, the scientific community is only beginning to understand the processes and pathways that enable successful agroecological transitions, in part because there are few contexts in which policy and market conditions support them (Miles et al., 2017). Here, we conducted an integrated ecological and socioeconomic assessment of farms transitioning from conventional tobacco monocultures to agroecological management of horticultural crops and livestock in southern Brazil. Our study is the first to show how farm management practices affect income and working conditions on farms at different stages of agroecological transition, in the context of a farmer network and supportive institutional environment in southern Brazil.

Theoretical frameworks of agroecological transitions have now existed for multiple decades (e.g., Hill and MacRae 1996). A large body of work summarized by Gliessman (2014) conceptualizes agroecological transitions as processes with five stages: (1) input efficiency, (2) input substitution, (3) system redesign, (4) formation of alternative food networks, and (5) construction of a new global food system. The first three of these occur at the agroecosystem level, the primary focus of the present study, whereas the final two stages necessarily include the entire food system. Input substitution and efficiency are considered "incremental" but necessary shifts toward sustainable food systems, while system redesign, alternative food networks, and developing a new food system are "transformational" (HLPE, 2019).

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[2] This chapter was written with co-authors Hannah Wittman and Jennifer Blesh. The published version can be found in the bibliography as Stratton et al. 2021b.

Practically, studying agroecological transitions requires the operationalization of concepts and indicators that encompass the management practices, social dynamics, and ecological innovations that together represent the field of agroecology (Wezel et al., 2009, 2020). As a science, agroecology applies ecological principles to agricultural systems to enhance biodiversity and ecosystem functions, with potential long-term benefits for soil fertility and productivity (Kremen et al., 2012; Gliessman, 2014). Increasing crop diversity on farms supports multiple ecological functions such as nutrient cycling and beneficial species interactions that contribute to the success of agroecological transitions (Isbell et al., 2017; Dainese et al., 2019). The effects of diversification on ecosystem services tend to be magnified when multiple practices are combined, such as by integrating mixed crop-livestock systems and cover cropping on a farm (Beillouin et al., 2019; Rosa-Schleich et al., 2019). Diverse crop rotations may also improve resilience on farms with adverse environmental conditions by increasing agroecosystem functioning, reducing reliance on a few staple crops, and balancing food availability throughout the year and over multiple growing seasons (Lin, 2011; Bowles et al., 2020).

In addition to ecological processes, agroecology emphasizes social transitions (e.g., through changing practices, farmer learning networks, and supportive social movements) that must also occur for long-term changes toward agricultural sustainability to take place (Mier et al., 2018; Ollivier et al., 2018). While no comprehensive list of socioeconomic indicators for agroecology has been developed, a recent review identified 13 main socioeconomic themes relevant to agroecology, including environmental equity, financial independence, market access and autonomy, sustainability and adaptability, and partnership between producers and consumers, among others (Dumont et al., 2016a). Each theme reflects agroecology’s valuation of self-governance and collective mechanisms for change, distinguishing agroecology from other agricultural paradigms that focus on profitability as a sole measure of socioeconomic success. More recently, Wezel and colleagues (2020) defined 13 agroecological principles that encompass ecological, social, and economic aspects of food systems and their importance for agroecological transitions, complementing the 10 elements of agroecology recently defined by the Food and Agriculture Organization of the United Nations (FAO) (Barrios et al., 2020). While both Dumont et al.’s themes and Wezel et al.’s social and economic principles apply at multiple levels of the food system, many of them have yet to be operationalized in agroecology research (D’Annolfo et al., 2017).

Only recently have empirical tests of processes and outcomes of agroecological transitions begun to emerge. The majority of studies comparing farms as they transition to sustainable agriculture tend to use a typologies approach based on level of market orientation (Kansiime et al., 2018) or specific farmer values (Teixeira et al., 2018), rather than ecologically-relevant indicators of farm management practices (Petit and Aubry, 2016; Dupré et al., 2017). However, process-based and mechanistic approaches to analyzing farm transitions may complement larger-scale studies and provide in-depth understanding about how specific practices, phases, and pathways influence ecological and socioeconomic outcomes (Lamine and Bellon, 2009; Mawois et al., 2019). A focus on the mechanisms and rate of change is also needed to understand the short-term, often negative, “transition effect” on productivity and profitability, which can discourage farmers from beginning or continuing to transition without adequate support (Martini et al., 2004; Lamine and Bellon, 2009).

Indeed, social and economic support systems for agroecology remain the exception rather than the rule. Agroecological farms receive less public and private financial support and investment than their conventional counterparts (Miles et al., 2017), demonstrating that the current agricultural technological regime has a high degree of lock-in (Geels, 2002; Vanloqueren and Baret, 2009). Without access to stable markets, knowledge, financial incentives, and other resources, agroecological transitions can be unattainable for many farmers (Blesh and Wittman, 2015; Guerra et al., 2017; Valencia et al., 2019). Agroecological transitions also face the challenge of structural changes in farms’ ecological and livelihood complexity, which are necessary to shift from a simplified production system to one with increased crop diversity (Vanloqueren and Baret, 2009).

When farmers have access to institutional supports, agroecological transitions may also be more likely to provide economic benefits, for example, through price premiums for certified produce and reduced input costs (Bowman and Zilberman, 2013; Valencia et al., 2019). Numerous global studies have compared the ecological and economic outcomes of organic and conventional agriculture, but most focus on simplified metrics of economic success, such as yield, and many find a "yield gap" between organic and conventional management (Seufert et al., 2012; Smith et al., 2020). Still, other meta-analyses have found positive relationships between farm diversification practices and yield (Ponisio et al., 2015; Dainese et al., 2019). Such global comparisons tend to capture a wide range of practices on organic farms, not all of which are necessarily agroecological, which may contribute to their variable results. Furthermore, they neglect the mechanisms by which diversification, and agroecological transitions specifically, occur. For these reasons, farm-level indicators of ecological management that reflect changes in important ecosystem processes over time are needed to better understand both social and ecological outcomes of agroecological transitions.

In addition to the need for precise indicators of ecological management practices, we also know relatively little about relationships between ecological management and socioeconomic outcomes in cases where markets, incentives, and farmer networks support diversified agriculture (D'Annolfo et al., 2017; Valencia et al., 2019). Recent frameworks have been developed to summarize socioeconomic themes important to agroecological transitions (Dumont et al., 2016a; Wezel et al., 2020) and to assess the sustainability of working conditions in agriculture (Dumont and Baret, 2017). However, such indicators are rarely evaluated in agroecology and other sustainable agriculture studies (D'Annolfo et al., 2017; Malanski et al., 2019); even fewer studies test socioeconomic outcomes on working farms (as opposed to experimental stations) or consider farmer perspectives on agroecological transitions (D'Annolfo et al., 2017).

Our study examines how farm management practices and socioeconomic conditions differ across a purposively selected gradient of farms transitioning from conventional tobacco to diversified agroecological production. We operationalize theoretical principles from prior frameworks (Dumont et al., 2016a; Dumont and Baret, 2017; Wezel et al., 2020) to understand how farm income and working conditions, both important socioeconomic outcomes, vary across stages of agroecological transition, under enabling conditions of strong institutional and market support (Table 2.1). We specifically sought to study agroecological transitions in a region where farmers have access to knowledge, resources, and diverse markets to support their transitions, motivating our extreme case sampling approach (Patton, 2014). In-depth interviews across a gradient of transitioning farms enable increased understanding of how different stages of transition influence socioeconomic outcomes, as well as ecological ones, based on farmers' experiences. When analyzed through the lens of qualitative causal explanation (Maxwell, 2004, 2012), case studies are valuable for suggesting causal processes and mechanisms of change in a specific social-ecological context (Magliocca et al., 2018). Results of our case study could support farmer decision-making and provide incentives for more risk-averse farms to undertake agroecological transitions under similar social and environmental conditions. Identifying key contextual factors or farm-level leverage points that support transitions could also aid policymakers in designing appropriate programs and incentives to support farmers as they transition.

Using an in-depth case study of farms transitioning from conventional tobacco production to agroecological management in southern Brazil (Figure 2.1), we asked the following research questions: (1) To what extent do farms' management practices at different stages of transition align with specific ecological indicators? and (2) How does transition stage influence income and working conditions on farms undergoing agroecological transitions?

Figure 2.1. Representative fields from farms at different stages of agroecological transition. Bottom: Farmers prepare to plant this steep, conventional tobacco field following a fallow. Middle: This farm in transition still plants mostly monoculture staple crops (such as yams, pictured here) but is adding perennials like banana to diversify its cropping system. Top: Agroecological farms use transformative practices such as intercropping and agroforestry, pictured here.

2.2 Materials and methods

Study area, site selection, and sampling approach

The state of agroecology in Santa Catarina, Brazil

Diversified family farms predominate in the agricultural landscapes of Santa Catarina, a state in southern Brazil (Figure 2.2). Agriculture in the region is highly influenced by its history of family farmer immigration from Europe and its steeply sloping terrain (Wildner et al., 2004; Wolford, 2010). Both erosion from agricultural land uses and continued deforestation of the native Atlantic Forest biome have historically reduced the state's soil fertility. Despite its difficult agricultural conditions, Santa Catarina farms are highly productive, yielding 13% of Brazil's national agricultural output on just 1% of its total land area (Wildner et al., 2004). Family farmers in the region produce horticultural and staple crops for both home consumption and sale to local, regional, and export markets. Due to steep topography and high rainfall in the region, the use of heavy machinery, frequent tilling, and high agrochemical inputs typical of conventional agriculture contribute to soil erosion, acidification, nutrient loss, and losses of soil organic matter (SOM) on short time scales (Primavesi, 1979). In the past several decades, declining soil fertility and crop yields have driven the state's agricultural extension organization, EPAGRI, along with scientists and other stakeholders, to prioritize research on sustainable management alternatives (da Costa et al., 2017).

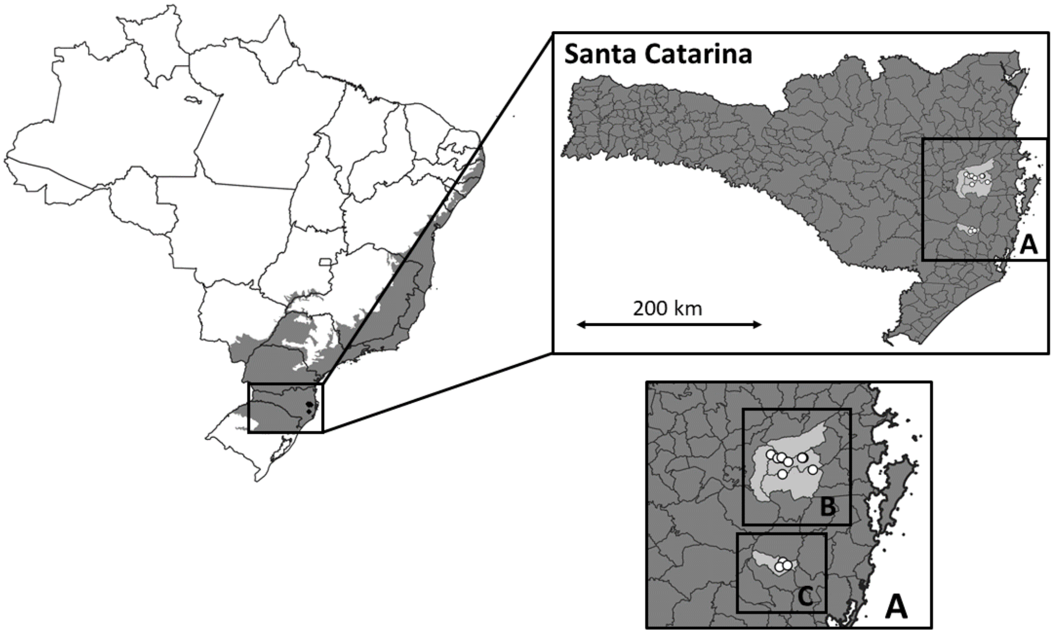

Figure 2.2. Brazil with the state of Santa Catarina (inset). A: Study region in eastern Santa Catarina. B: Major Gercino region. C: Santa Rosa de Lima region. Shading denotes the prior extent of the Atlantic Forest biome, much of which has now been deforested for agricultural land uses.

Santa Catarina is a stronghold for the field of agroecology as a practice, a movement, and as a science (Wezel et al., 2009). The state has a high prevalence of farmer networks, social movements, non-profits, and research and other public institutions dedicated to advancing agroecological management. Farmers in Santa Catarina can also access two government-mediated markets with price premiums for organic or agroecologically certified produce: PNAE, the federal school lunch program, and PAA, the now-defunded federal food acquisition program. Though certified organic and agroecological farmers make up only 1% of all farms in the state, the number of certified farms has tripled over the past decade (Marcondes, 2018). In 2019, certified agroecological and organic farmers in Santa Catarina numbered 1,275, with 700 more in transition (EPAGRI, 2019); both figures are likely underestimates, as farmers commonly employ agroecological practices without certification. Certification in Brazil can occur one of two ways—through third-party audits, or through participatory guarantee systems.

Participatory guarantee systems offer a lower-cost option than third-party certification for farms transitioning to organic management. In this model, farmers do the work of certifying one another, and the network offers built-in opportunities for knowledge and resource-sharing related to agroecological management (Guerra et al., 2017). The main cost of the process is time, as participation in meetings and farm verification events is mandatory and enables the continued functioning of the system. While there are numerous participatory guarantee systems for farmers in different regions of Brazil, the predominant system in southern Brazil is Rede Ecovida [Ecovida Network of Agroecology], an agroecological network made up of farmers, consumers, and supporting institutions (Rede Ecovida de Agroecologia, 2004). Rede Ecovida spans three Brazilian states—Paraná, Santa Catarina, and Rio Grande do Sul—and partners with other farming organizations across Brazil. The network is composed of 340 farmer groups totaling about 4,500 family farms and 20 NGOs across southern Brazil. Approximately 1,000 farms in Santa Catarina are certified through Rede Ecovida. To retain their certification, farmers must use organic nutrient sources and other ecological management practices, avoiding synthetic fertilizers and pesticides, to maintain or improve their soil fertility and crop yields (Rede Ecovida de Agroecologia, 2004).

Farm selection and sampling approach

We conducted 13 months of intensive, interdisciplinary fieldwork in Santa Catarina between 2017 and 2020, combining qualitative and quantitative data collection methods. We worked with the agroecology non-profit CEPAGRO (Center for the Study and Promotion of Group Agriculture), a Rede Ecovida partner organization based in Florianópolis, Santa Catarina, to identify farms interested in participating in a multi-year, integrated social-ecological study on diversification practices. The case study sample was selected from a group of farm households participating in a two-year field experiment testing effects of crop diversification on soil fertility, yields, and crop quality (Chapter 3). Farmers in our sample represent ecological innovators within their local agricultural context, and all of them have experience with the agroecological participatory certification network Rede Ecovida, with the agroecology nonprofit and member organization CEPAGRO, or both. We used a purposive sampling approach to understand the processes and mechanisms of transitioning to agroecological management in a context in which farmers have access to knowledge and resources to support their transitions (Patton, 2014).

Descriptive, place-based narratives can be used to understand how social and environmental contexts interact with specific decisions or mechanisms to generate the observed effects (Maxwell, 2012; Magliocca et al., 2018). To analyze our interview data, we used a qualitative causal explanation approach (Maxwell, 2004) with extreme case sampling of innovative farm households, all of which had years of institutional support and exposure to ecological management techniques (Patton, 2014). Our study design aimed for in-depth analysis, including multiple years of contact between researchers and each participating household, exhaustive farm management surveys paired with semi-structured qualitative interviews, and informal conversations with dozens of additional transitioning farmers. This rich qualitative data, paired with purposive sampling of farms across three stages of transition, provided the means to study how farmers perceived the causal mechanisms and effects of agroecological transitions.

After conducting preliminary management interviews and analyzing soil samples collected from 20 farms in eastern Santa Catarina, we selected 14 farms to participate in our study, which ran from February 2018 to June 2020. A sample size of 14 households within this methodological approach was sufficient to reach "saturation," or the point in qualitative interview analysis at which no new information or themes are garnered from additional data (Guest et al., 2006). Our approach to qualitative causal explanation was comparative: all of the farms had similar management histories in conventional tobacco production, similar climate and soil types, and access to similar resources through agroecology institutions, but they had distinct transition stages and years of experience with agroecological management (Maxwell, 2004). Comparison between transition stages (conventional, transition, and agroecological) generated a counterfactual for what agroecological farms could look like if they were still conventional. We also analyzed individual farmer interviews to understand how each farming household viewed the evolution of their management practices and socioeconomic outcomes over time and at different stages of transition.

All farms were located in the same climatic region, with subtropical weather patterns and rainfall averaging 1,500-1,700 mm per year (Wrege et al., 2012; Marinho et al., 2020). Similarly, farms were selected in a single soil microregion, meaning soil types share parent materials and physiochemical characteristics. Soils throughout the region are associations of alic cambisols and red-yellow alic podzols (EMBRAPA, 2004). Both soil types have low pH, low nutrient availability, and moderate to low moisture availability. Phosphorus (P) deficiency and aluminum toxicity are common agricultural problems in the region. Within this environmental context, farms were chosen to represent a gradient of transition stages, with four *conventional* farms, five farms in *transition* to agroecological management (0-5 years certified organic through Rede Ecovida), and five established *agroecological* farms (>5 years certified). We used survey and interview data from farms at each of these transition stages to assess ecological management practices and socioeconomic outcomes.

Data collection

Our ecological field experiment examined the effects of cover cropping and intercropping on soil nutrient cycling and crop yields on farms across three transition stages (Chapter 3). Over the course of the ecological experiment, we collected interview data and field observations on farmers' management practices, socioeconomic outcomes, and experiences with the process of transitioning to agroecological management. We conducted household surveys at the beginning, mid-point, and end of the study, which we paired with semi-structured interviews at two time-points to complement quantitative information with rich, descriptive data about the transition process (Maxwell, 2004). The main period of data collection took place in May 2019 and combined structured surveys and semi-structured interviews with farm households, which together lasted two to three hours.

Management and socioeconomic surveys included the following sub-sections: (a) farm characteristics and demographics, (b) farm land use and field characteristics, (c) crop rotations by field, (d) management and inputs by crop type, (e) farm labor patterns by worker, (f) household earnings (markets, yields and prices of principal crops, agricultural and non-agricultural household income and expenses), and (g) farmers' evaluation of the ecological experiment. Semi-structured interviews focused on the concept of transition, asking farmers what changes in crop selection, inputs, income and expenses, yields, and work quantity and quality had occurred since officially beginning the "organic" transition process, or in the last ten years, if conventional. We also asked farmers what key sources of information or learning experiences they relied on during the transition. Questions were designed to be open-ended to elicit novel responses. To corroborate original information on household earnings and labor collected in May 2019 and reduce recall decay (Beegle et al., 2011), we collected additional socioeconomic data from each farm in October 2019.

Participant observation was a mainstay of our study. The lead author cohabitated with farmers and shared agricultural and domestic work during four field campaigns over two years, each of which involved four or more day-long visits to experimental plots on each farm. We also performed agricultural tasks with farmers as participant observation for one day per farm. In addition to work in the field with farmers, we participated actively in local, regional, and network-wide meetings for Rede Ecovida. To triangulate our results and account for researcher bias, we regularly held "member checks" with farmers and employees at CEPAGRO, enabling us to adapt our theorized relationships based on the perspectives of the group of study (Maxwell, 2004).

We completed two rounds of thematic coding of interview transcripts using NVivo text coding software (QSR International). Qualitative observations and experiences of farmers were used to understand the mechanisms driving quantitative results. All interviews were conducted in Portuguese by the lead author, and direct quotes used in the text were translated to English following thematic coding.

Indicator framework for agroecological transitions

Ecological management indicators

The 14 farms in our study spanned a wide diversity of practices, both within and across the three stages of transition (i.e., conventional, transitioning, and agroecological). To more precisely characterize ecological management on farms, we developed four indicators to measure on all farms in the sample based on known links between agroecosystem management or structure and ecosystem functions across transitions (Shennan, 2008; Wezel et al., 2014): (1) crop and livestock diversity (Hill, 1973; Jackson et al., 2007), (2) continuous soil cover (Tonitto et al., 2006; King and Blesh, 2018), (3) ecological nutrient management (Drinkwater and Snapp, 2007a; Drinkwater et al., 2008), and (4) ecological pest management (Letourneau et al., 2011; Kremen and Miles, 2012) (Table 2.1). In addition to overall crop diversity, the specific addition of perennials and other forms of continuous living soil cover is known to exert proportionally large effects on soil fertility (King and Blesh, 2018) and is of particular importance in regions vulnerable to erosion. Complementing continuous soil cover, ecological nutrient management consists of a suite of practices that reduce use of external nutrient inputs, manage crop and livestock diversity to cycle and retain nutrients, and build soil organic matter. Ecological pest management employs intentional management of crop and livestock diversity and other methods of biological control to modulate disease and pest populations. Each of our indicators aligns with broader ecological knowledge and also corresponds with one or more agroecological principles from the recent literature, including biodiversity, soil health, recycling, and input reduction (Wezel et al., 2020). Indicators were quantified using data from management interviews and field observations. Detailed information about indicator quantification can be found in Appendix 1.

Table 2.1. Indicators of ecological management, financial independence, and working conditions on farms.

Indicator	Definition	Measure
Ecological Management		
(1) Crop and livestock diversity	Number of crop and livestock species (including fish) produced on a farm, weighted by area in production	Simpson's diversity (1-*D*, where $D = \sum p_i^2$, and p_i is the proportional abundance of species *i*)
(2) Continuous soil cover	Presence of permanent or semi-permanent vegetative cover on agricultural lands to stabilize soil, reduce nutrient losses, and build organic matter	Proportion of managed farm area in perennials, or annuals with cover crops during fallow period
(3) Ecological nutrient management	Farm management that increases internal nutrient cycling and maintains soil nutrient pools with organic matter inputs to achieve optimal yields	Proportion of farm under ecological soil fertility management (e.g., application of compost, manure, cover crop biomass)
(4) Ecological pest management	Farm management that increases biodiversity and stability of pest populations; prevention or use of biological control for outbreaks	Proportion of farm under ecological weed, insect & disease management
Financial Independence		
(1) Net agricultural income (per capita)	Agricultural income exceeding operating costs (e.g., farm gross value added), on a per person basis	Annual gross agricultural income minus production costs divided by the number of household members
(2) Net off-farm income (per capita)	Off-farm income exceeding expenses, on a per person basis	Annual gross off-farm income minus expenses divided by the number of household members
(3) Net total income (per capita)	Combined agricultural and off-farm income exceeding operating costs, on a per person basis	Annual gross household income minus expenses divided by the number of household members
(4) Market access	Number of different types of marketing channels accessed	Number of market types that contribute to farm annual income (out of 8 types)
Working Conditions		
(1) Agricultural labor hours (per capita)	Total time spent on agricultural activities (e.g., field-based, processing, marketing) on a per-worker basis	Number of hours spent on agricultural activities per week or year divided by the number of workers
(2) Off-farm labor hours (per capita)	Total time spent on off-farm activities on a per-worker basis	Number of off-farm working hours per week or year divided by the number of workers
(3) Total labor hours (per capita)	Total time spent on agricultural and off-farm activities on a per-worker basis	Number of working hours per week or year divided by the number of workers
(4) Occupational health	Farmer-reported safety of agricultural work and associated health conditions	Qualitative description
(5) Work quality	Farmer-reported level of satisfaction with work, including mental, emotional, and physical wellbeing	Qualitative description

Socioeconomic outcome indicators

Drawing on recent work highlighting emerging socioeconomic themes relevant to agroecology by Dumont and others (2016, 2017), we evaluated four indicators of *financial independence* and five indicators of *working conditions* across stages of agroecological transitions (Table 2.1). We conceptualize *financial independence* as a function of both income and level of perceived control over farm economic and technical decision-making. To assess each farm's *financial independence* (Dumont et al., 2016a), we measured net income from agricultural and off-farm work (using three indicators, described below), as well as access to diverse markets to sell produce (Dumont et al., 2016a; Roest et al., 2018; Valencia et al., 2019). Net agricultural income was calculated as earnings from agricultural activities minus farm operating costs and was divided by the number of household members for the per capita value. Net off-farm income was calculated as the difference between gross off-farm income and associated expenses, also on a per capita basis. Net total income was calculated as the sum of gross household income from all agricultural and off-farm sources, minus the sum of all agricultural and non-agricultural household expenses (e.g., gasoline for car travel, electricity costs, cell phone use, etc.) per capita. We use the term "net agricultural income" with several caveats; namely, we do not account for depreciation of agricultural equipment or for in-kind contributions from home consumption of agricultural products (Grosh and Glewwe, 2000).

Based on the family farming context and primary themes farmers emphasized in interviews, we operationalized five indicators of working conditions: occupational health, time at work (per capita labor hours, divided into agricultural, off-farm, and total hours), and the intrinsic benefits of work (work quality/job satisfaction). Occupational health refers to the level of physical and mental wellbeing at work (Dumont and Baret, 2017). Total time at work includes all working hours associated with farming, including production, processing, marketing and sales (including agritourism), and paperwork (agricultural working hours), in addition to any paid off-farm labor hours. Work quality is a measure of worker-reported wellbeing, here representing farmers' interest in work and expressions of satisfaction or contentment in their day-to-day activities (Harrison and Getz, 2015; Timmermann and Felix, 2015; Dumont and Baret, 2017). Each of our socioeconomic indicators relates to overarching agroecological principles, with financial independence tied to economic diversification and sustainable land and natural resource governance, and working conditions related to concepts of social values, fairness, and participation (Wezel et al., 2020). Additional information regarding the quantification of socioeconomic indicators is included in Appendix 1.

2.3 Results and discussion

Farm characteristics

Participating farms were located in two municipalities with similar climatic and soil conditions—Major Gercino and Santa Rosa de Lima (Figure 2.2) (EMBRAPA, 2004). Across the state, 13% of farms produce conventional tobacco (Marcondes, 2018). While tobacco remains an important crop in Major Gercino and surrounding municipalities, Santa Rosa de Lima has a higher proportion of agroecological farms, an organic food processing and marketing cooperative (AGRECO), and an agritourism organization, Alcolhida da Colônia (EPAGRI, 2019). Across the sample, farm size ranged from 12 to 76 ha and cultivated area ranged from 3 to 48 ha (Table 2.2). Agroecological farms (mean=14 ha) tended to be slightly smaller than conventional farms (mean=30 ha), but there was substantial variation within groups. Principal marketed crops followed regional patterns, including notable production of tobacco (on conventional farms), grapes, banana, and honey in Major Gercino and diversified fruit and vegetable products in Santa Rosa de Lima (Marcondes, 2018). None of the transitioning or agroecological farms continued to grow tobacco after joining Rede Ecovida; farmers perceived that nicotine poisoning from harvesting green tobacco ("green tobacco sickness"; Fotedar and Fotedar, 2017) was even more severe in organic tobacco production, and they preferred to avoid it. All farmers in our sample had at least a decade of experience in agriculture (mean=29 years) and came from farming backgrounds, and all farms except one had a history of chemical-intensive tobacco farming. The remaining farm had a history of low-input subsistence agriculture, primarily producing staples such as cassava, beans, yams, and maize. The three farmers in our sample from Santa Rosa de Lima participated in value-added food processing through AGRECO and agritourism through Acolhida da Colônia.

Table 2.2. Descriptive characteristics of conventional, transitioning, and established agroecological farms in the case study in Santa Catarina, Brazil.

Farm ID	Transition stage	Region	Number of house-hold members	Mean age in house-hold (years ± SD)	Mean education level in house-hold (years ± SD)	Yrs of farming experience (house-hold heads)	Years cert. organic	Highest earning crop	Prod. of primary crop (kg/year)	Farm size (ha)	Cult. area (ha)
1	Conventional	Major Gercino	6	39 ± 17	9 ± 4	18	0	tobacco (dried)	11,250	57	28
2	Conventional	Major Gercino	6	44 ± 18	5 ± 3	15	0	tobacco (dried)	10,500	36	22
3	Conventional	Major Gercino	6	53 ± 13	7 ± 4	35	0	green bean (canned)	10,300	61	48
4	Conventional	Major Gercino	5	25 ± 15	9 ± 3	35	0	tobacco (dried)	8,000	32	21
5	Transition	Major Gercino	5	54 ± 25	7 ± 6	22	0	banana	4,000	45	29
6	Transition	Major Gercino	2	38 ± 6	10 ± 0	16	3	banana	9,000	68	29
7	Transition	Major Gercino	7	34 ± 14	9 ± 4	33	3	tomato	5,500	31	19
8	Transition	Major Gercino	4	48 ± 24	10 ± 4	60	5	honey	1,000	60	30
9	Transition	Major Gercino	5	40 ± 24	8 ± 4	32	5	grape	12,000	76	28
10	Agroecological	Major Gercino	3	36 ± 21	8 ± 1	38	7	banana	12,000	25	16
11	Agroecological	Santa Rosa de Lima	2	38 ± 18	14 ± 3	24	8	honey	2,000	12	3
12	Agroecological	Santa Rosa de Lima	4	35 ± 15	15 ± 2	14	9	blackberry	1,000	19	4
13	Agroecological	Major Gercino	5	27± 20	6 ± 2	29	12	grape	30,000	28	25
14	Agroecological	Santa Rosa de Lima	3	43 ± 25	9 ± 1	35	20	cassava (flour)	700	42	23

All farms had access to multiple sources of knowledge and technical training around agroecology. Over the last decade, farmers participated in trainings from multiple institutions, including our partner organization CEPAGRO (Major Gercino), the state agricultural extension agency EPAGRI (Major Gercino and Santa Rosa de Lima), tobacco companies such as Souza Cruz (Major Gercino), and university researchers (Santa Rosa de Lima). CEPAGRO staff, who have worked with many farmers in these regions for the past 30 years, consider the farmers in this study to be leaders and conservation innovators in their respective transition stages. Farmers' support networks, however, differed between conventional farmers and the two agroecological stages. Tobacco producers received most of their crop advising from tobacco company representatives, whereas transitioning and agroecological farmers relied on regular support from other farmers and organizations in Rede Ecovida. Established and transitioning agroecological farmers attended monthly Rede Ecovida group meetings in their respective municipal regions, both of which were part of the Coastal Santa Catarina Nucleus, which holds bi-annual meetings open to all participating farmers.

Family participation in agriculture is a prominent feature of farming culture in Santa Catarina and has been cited as one factor that promoted strong social movements in the region, such as the *Movimento dos Trabalhadores Sem Terra* (MST) and Rede Ecovida. This tendency held true for farms in our case study. Farm household size ranged from two to seven, with a median value of five. Although five farmers shared narratives of local youth migrating to urban centers, four of these cases involved young people moving to the city for higher education or to seek employment and then returning to support and build the family farm. This trend opposes Santa Catarina's increasing emigration from rural areas; the state's farming population reached its peak in 1985 and has since fallen by 56% (Marcondes, 2018). Agroecological farmers expressed the hope that their diverse crop and market structures and youth involvement in Rede Ecovida could reverse this trend and keep their children and relatives on the farm.

Ecological management across transition stages

Indicators of ecological management tended to increase from conventional to agroecological farms, as expected. Though 12 of the 14 farms in the sample had diversified home gardens for household consumption, both total and marketed crop richness increased from conventional to agroecological farms (Figure 2.3, Figure 2.4). Mean livestock richness was also higher on agroecological farms than on conventional or transitioning farms. These results are perhaps unsurprising, given the tenets of agroecology required for participatory certification through Rede Ecovida. Recommendations go beyond typical organic certifications by requiring not only elimination of synthetic fertilizers and pesticides, but also increased crop diversity, crop-livestock integration, use of perennials, organic nutrient sources, and ecological pest control methods, such as biocontrol and push-pull techniques (Rede Ecovida de Agroecologia, 2004;

Cook et al., 2007). It is important to note the higher variability in ecological management practices on farms in transition, which includes farms with up to 5 years of organic certification.

Farmers' management practices mapped closely onto agroecological transition stages of input efficiency, input substitution, and system redesign (Gliessman, 2014). Conventional farmers diversified their crop rotations to increase the efficiency of synthetic input use; farmers in transition and in the early years of organic certification (0-5 years certified) relied on input substitution, substituting purchased organic inputs for synthetic pesticides and fertilizers; and established agroecological farmers (>5 years certified) used transformative practices such as mixed crop-livestock systems and agroforestry to redesign their systems and reduce labor and input costs.

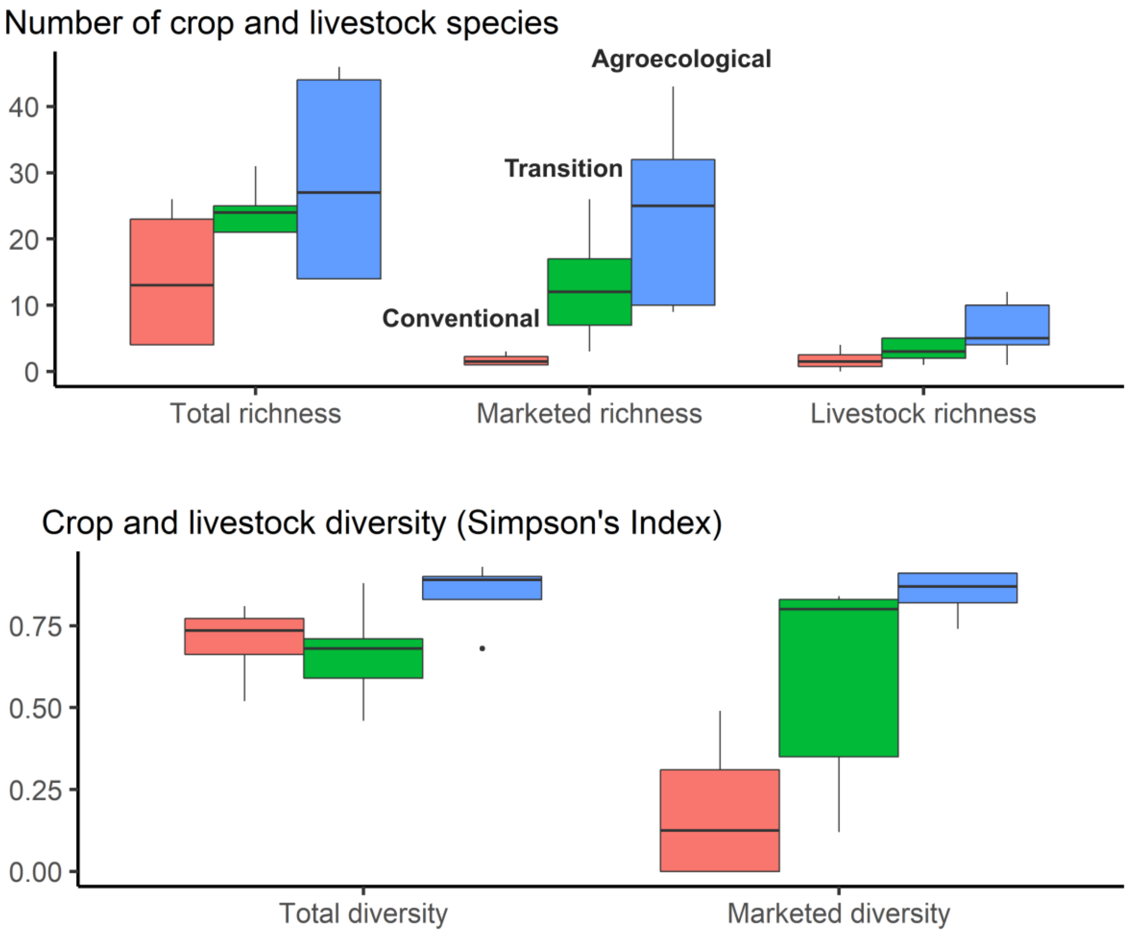

Figure 2.3. Farm-level crop and livestock diversity across agroecological transition stages shown as species richness (top) and Simpson's diversity index (bottom). Total crop and livestock species produced per farm are shown on the left and marketed species are shown on the right.

Crop and livestock diversity

Marketed crop and livestock diversity was highest on agroecological farms (mean=24 crops, sd=15), followed by farms in transition (mean=13, sd=9), and finally conventional farms (mean=2, sd=1) (Figure 2.3). While half of the conventional farmers in our sample were "diversified" tobacco producers, planting rotations of tobacco, maize, and arracacha (*Arracacia xanthorrhiza,* a biennial root vegetable in the carrot family) cash crops with grass-legume cover crop mixtures in between, the other half planted fewer crops in rotation and irregularly cover cropped with black oat (*Avena strigosa*). Conventional farmers cited tobacco companies as a main influence in their decision to increase their crop rotational diversity from what were previously tobacco monocultures. Farmer 1 asserted:

> Today even the tobacco companies have changed their point of view. They want a well-diversified farmer, too. They prefer a farmer that can stay on the land – that's an advantage for them. They don't really want some broke farmer depending on them because it ends up generating a loss for them at the end of the day. [The farmers] won't be able to invest in the equipment [the companies] require.

Farms in the early stages of agroecological production had the most variation in marketed crop and livestock diversity, as well as other ecological management indicators (Figure 2.4). Marketed crop richness ranged from 3 to 26 crops on transitioning farms, depending on their management histories. These farmers managed the agroecological transition with an input substitution approach—substituting a conventional input for an organic one, without changing the structure or function of the agroecosystem (Rosset and Altieri, 1997; Duru et al., 2015a), and several established agroecological farmers described going through a similar phase during their transitions. For example, one agroecological farmer reflected on all the products they used to purchase in the early years of the transition, explaining,

> We bought chicken litter, even sawdust, … for soil cover. We would plant a row of turmeric and leave that bare space in between. Then we weeded that area and left it all exposed, so we had to buy sawdust to put between the crops. Later, we started to put beans and other crops between rows, so the rain wouldn't compact the soil so much. … So, that helped and we didn't need to do so much anymore, since the bean leaves fell there and covered the soil, helping out the situation.

Marketed crop richness was high on established agroecological farms (9-43 species) (Figure 2.3). Farmers in this group relied on several forms of agroecosystem redesign, including use of diversified agroforestry systems (n=3 farms), integrated crop-livestock systems (n=3), and intercropping with annuals and perennials (n=5, 100%), as well as livelihood or market diversification (n=5). Nearly all agroecological farmers described this transformation of their farming systems from monoculture tobacco with a few subsistence crops to diversified, complex agroecological cropping systems as a learning process with various phases. Similar to prior findings (Lin, 2011; Blesh and Wolf, 2014), farmers in our study described a gradual shift from reliance on input substitution toward their realization that crop and livestock diversity could be used as a tool to reduce agricultural expenses and labor, while also increasing the resilience of their farm to market and biophysical shocks. For example, one agroecological farmer spent 17 years farming tobacco before transitioning to diversified vegetable production and has now been certified in Rede Ecovida for 12 years. The farm has a crop-livestock system with dairy cows grazed under perennial grapevines, annual intercropping, and consistent use of grass-legume cover crop mixtures during fallows. Explaining the farm's change in approach over the transition, this participant said,

> [Our input use] changed a ton because in the beginning we thought that the cost [of transitioning] would be high because you had to buy organic fungicides and insecticides, let's say, but today we can see that we no longer need those things on our farm. In reality, you have to diversify. Good soil keeps the plants strong, so you don't need to be dumping on insecticide and those things.

Continuous soil cover

Due to the importance of erosion prevention in this hilly region of Santa Catarina, soil cover was high across all stages of transition (Figure 2.4), although agroecological and transitioning farms had slightly higher proportions of their farms under perennials and cover crops than conventional farmers. Conventional farms maintained 65% of their farmland with continuous soil cover on average. Responding to falling yields and growing farmer indebtedness (according to farmers across transition stages who had grown tobacco), tobacco companies now recommend that farmers rotate their cash crops with cover crops to reduce soil erosion and disease pressure, build soil organic matter, and maintain tobacco yields over time. Conventional farmer 4, who had the lowest marketed crop and livestock diversity (a simple tobacco-silage corn rotation and several beef cattle) of any farm in the case study, had just begun using cover crops in an effort to rebuild his soil, as he reported he had been increasing his chemical inputs with diminishing returns, and it was time to make a change to increase his input use efficiency.

Most farms in transition had high levels of continuous soil cover (mean=94%, sd=4), but this number represents a high proportion of monoculture eucalyptus plantations (mean=27% of perennials, sd=27), which are grown as "reforestation projects" supported by government incentives and are also used to heat tobacco drying ovens. Conventional farmers also had comparably high proportions of eucalyptus (26% of perennials), whereas agroecological farms had fewer areas dedicated to tree plantations (10% of perennials). Agroecological farms had a high proportion of their farms under continuous soil cover (mean=90%, sd=11), with large areas of diverse perennial agroforests and extensive use of cover cropping.

Ecological nutrient management

While conventional farms in the sample managed cover crops to recycle and supply nutrients on 40% (sd=40) of their land on average, transitioning (mean=73%, sd=35) and established agroecological farms (100%, sd=0) had higher mean proportions of their farms under ecological nutrient management, recycling organic residues into soil to build fertility, including use of on-farm sources of composted food scraps, manure, and cover crop biomass (Figure 2.4). Conventional farmers in the study stated that adding cover crops in rotation provided benefits for fertilizer input efficiency, building soil organic matter and soil nutrient-holding capacity over time, which follows from broader understandings of ecological nutrient management (Drinkwater and Snapp, 2007b). Tobacco farmers in our sample thereby reported reducing their synthetic N fertilizer use by 15-50% with cover crops.

The wide variability in ecological nutrient management strategies among farms transitioning to agroecological methods provides evidence that agroecological transitions are a process with distinct trajectories and timelines (Figure 2.4). This variation results from the tendency of transitioning farms to maintain distinct sections of their farm under conventional management, applying synthetic fertilizer to conventional areas that are not yet in transition to agroecological certification. Similarly, some farms in transition continued to apply herbicides, although because occupational health was a principal motivation for many farmers to transition, continued pesticide use during transitions was less common.

Because established agroecological farms had completely shifted production over to certified organic, they were obligated to rely solely on ecological fertilizer and pest control methods; as such, 100% of all agroecological farms' fields were under ecological management for both of those indicators. Agroecological farmers also emphasized the use of inputs from inside their farms, including taking advantage of manure, kitchen scraps, forest litter, and processing waste from value-added products (e.g., liquid extracted from cassava flour, grape peels from juice production) to make compost for their crops—all examples of ecological nutrient management.

Ecological pest management

Weed and insect pest pressure can increase during agroecological transitions, due to reduced tilling and sudden restrictions on chemical pesticide use. Conventional farmers still scored lower than other transition stages in ecological pest management, with 20-50% of their cultivated area managed without use of synthetic pesticides (mean=34%, sd=11) (Figure 2.4). In fact, conventional farmers stated that cover cropping increased their reliance on the herbicide glyphosate, which was used to kill cover crops prior to planting cash crops. Farms in transition (mean=96%, sd=8) and agroecological farms (100%, sd=0) used ecological pest control methods on nearly all of their managed land.

Farmers in the transition group regularly cited increases in labor due to the need to use manual rather than chemical forms of weed control, as has been shown in other systems reliant on hand-hoeing (Nyamangara et al., 2013). Some farmers in transition lamented the loss of herbicides as a tool to reduce labor difficulty and working hours. When asked if he had observed any environmental changes during the transition, one transitioning farmer commented jokingly that "the weeds are taller and there are more leaf-cutter ants!", indicating that pest pressure had increased, along with his household's workload. One older farming couple (transitioning farm 8) expressed a more complex understanding of herbicide use during the transition process:

> Husband: We maybe spent more on inputs [as conventional farmers], but the herbicide was easier. It was a little less work because you just put it on and it lasts much longer before the weeds come back.
> Wife: Except the land thins out – because it doesn't produce [biomass].
> Husband: That's the part where people are losing a lot, because people aren't careful, because what's an advantage in [herbicide use] also has its downsides. Because the land starts degrading. In a few years it won't give anything anymore.

By killing emerging weeds with herbicide and keeping soil bare, the farmers limited the living biomass on their fields and increased chances of soil erosion. Their quote reflects an ecological understanding of agriculture—that a farm field without soil cover and biomass inputs will lose fertility over time—but the farming couple did not go so far as to suggest alternatives to manual weeding or weed whipping as a substitute for herbicide, in keeping with the input substitution ideology. This pattern distinguished farms in transition from established agroecological farms, which focused on increased use of intercropping, diversified perennial systems, and animal integration as strategies to reduce weed and insect pressure and provision nutrients inside the farm.

Past research has found that farmers' management and labor outcomes can change as they develop skills with ecological methods for weed suppression, including techniques using crop competition (Mhlanga et al., 2016) and cover crops (Kruidhof et al., 2010; Navarro-Miró et al., 2019), which can reduce the need for hand-weeding and longer working hours. Many agroecological farmers saw increased biodiversity and practices like intercropping as less work-intensive ways to control pests and produce more, as agroecological farmer 11 described:

> The main motivation [to diversify] was to control diseases because I noticed that we get great production in intercrops. It is easier; we don't have to do so much work. The thing I've observed the most is that when one plant "likes" the other, it seems like it produces much more.

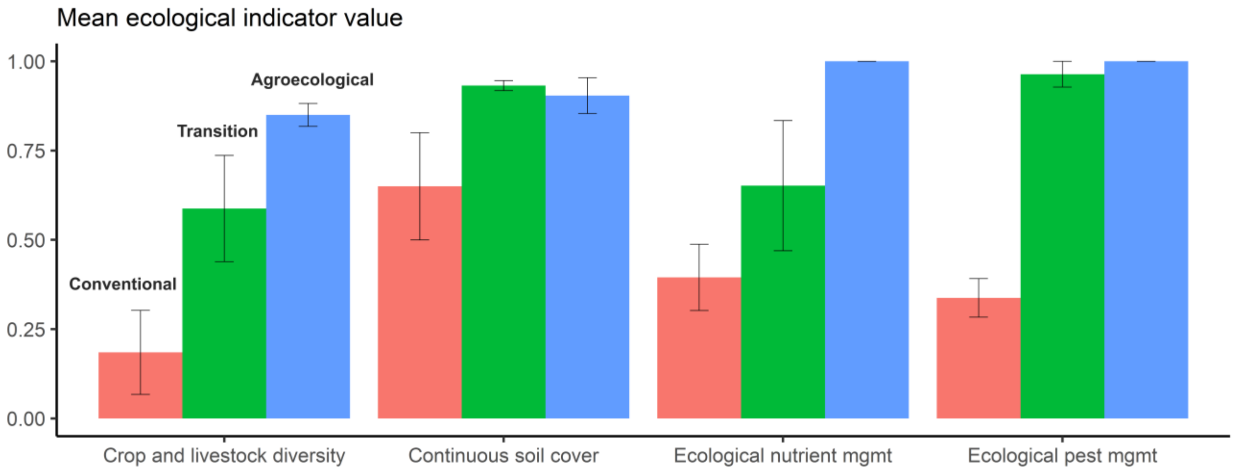

Figure 2.4. Farm ecological management indicators at different stages of agroecological transition (mean values with standard error). Four indicators of ecological management are shown: (1) marketed crop and livestock diversity (Simpson's diversity index), (2) proportion of farm under continuous soil cover, (3) proportion of farm under ecological nutrient management and (4) proportion of farm under ecological pest management. All indicators were calculated on a scale from 0 to 1, with 1 representing greatest alignment with agroecological principles, and 0 representing least alignment.

Socioeconomic outcomes across transition stages

As one conventional farmer stated, it is not enough for agriculture to be diversified; it must also make ends meet for the household. Prior studies have emphasized approaches to minimize tradeoffs between profitability and ecological functions on farms, primarily through price premiums and increased input efficiency (Crowder and Reganold, 2015; van der Ploeg et al., 2019). Our case study results indicated that over the course of agroecological transitions, use of system redesign approaches rather than input substitution contributed to improved socioeconomic outcomes. Using our indicator framework, we identified two overall socioeconomic benefits related to ecological management changes during agroecological transitions in the case study: increased *financial independence* and improved *working conditions* (Table 2.3, Figure 2.5).

Financial independence

Income

Agroecological farms demonstrated increased financial independence relative to conventional and transitioning farms (Figure 2.5). Net total income was highest on agroecological farms, moderate on transitioning farms, and lowest on conventional farms on a per capita basis (Table 2.3), while net household income overall (e.g., not accounting for household size) was lowest on transitioning farms (Appendix 1, Table A1.1). Net agricultural income (per capita) was similar between agroecological and conventional farms but lower on farms in transition. Transitioning and agroecological farms received 38 and 41% of their gross annual income from off-farm sources on average, representing livelihood diversification beyond agriculture, whereas conventional farmers received only 1%. Net agricultural income per cultivated hectare was also nearly 2.5 times higher on agroecological farms than on conventional farms due to similar earnings from smaller cropped areas. Our results align with the "transition effect" commonly observed on certified organic farms (Martini et al., 2004; Lamine and Bellon, 2009), as agricultural income tended to be lower on farms in the first five years of the transition. This analysis also demonstrates that established agroecological farms were able to achieve comparable levels of net agricultural income as conventional farms—along with additional socioeconomic benefits, described below—following a transition period.

Table 2.3. Mean values and standard deviations (SD) for each indicator of financial independence and working conditions by agroecological transition stage. Net agricultural and total income exclude monetary contributions from household self-provisioning and losses due to depreciation. Additional socioeconomic indicators can be found in Appendix 1, Table A1.1.

Indicator type	Indicator	Conventional		Transition		Agroecological	
		Mean	SD	Mean	SD	Mean	SD
Financial independence	(1) Net agricultural income (per capita)	$1,964	$1,351	$1,241	$1,403	$2,102	$1,874
Financial independence	(2) Net off-farm income (per capita)	$375	$750	$1,521	$1,461	$3,183	$2,247
Financial independence	(3) Net total income (per capita)	$2,339	$1,491	$2,762	$902	$5,284	$3,459
Financial independence	(4) Market access	2	1	3	2	3	1
Working conditions	(1a) Annual agricultural labor hours (per capita)	2830	684	2150	252	2234	590
Working conditions	(1b) Weekly agricultural labor hours (per capita)	54	13	41	5	43	11
Working conditions	(2a) Annual off-farm labor hours (per capita)	0	0	210	254	339	503
Working conditions	(2b) Weekly off-farm labor hours (per capita)	0	0	4	5	7	10
Working conditions	(3a) Annual total labor hours (per capita)	2830	684	2360	296	2573	920
Working conditions	(3b) Weekly total labor hours (per capita)	54	13	45	6	49	18
Working conditions	Number of workers	6	1	4	3	4	2
Working conditions	Number of household members	6	1	5	2	3	1

According to interview data, the mechanisms behind these outcomes at different transition stages related primarily to two changes in management on established agroecological farms: reduced synthetic input intensity (and corresponding savings on input costs) and increased diversity of marketed products. Diversification in agriculture has long been known to reduce variability in income and labor (Heady, 1952), supporting farmer livelihoods throughout the year. Reduced input intensity affected net agricultural income, whereas increased diversity affected the consistency of income and labor patterns, but both relate to the idea of "farming economically" by reducing input costs per output (van der Ploeg et al., 2019).

Conventional tobacco farms in our sample tended to have high gross annual agricultural incomes (mean=$66,248) but also high agricultural expenses (mean=$55,129), whereas both farms in transition and agroecological farms had much lower gross agricultural incomes (means=$10,078 and $12,975, respectively) but even lower farm expenses (means=$5,156 and $6,483) (Appendix 1, Table A1.1). This extreme difference results in part from the high levels of investment and inputs required for tobacco production for export, but it aligns with broader trends in global input-intensive agriculture, in which rising input costs and falling prices due to overproduction lead to a cost-price squeeze on farm incomes (Goodman and Redclift, 1991; Rosset and Altieri, 1997; Crews and Peoples, 2004). Tobacco farmers in the sample participated in a contract scheme with export-oriented tobacco companies, in which they purchased inputs (fertilizer, agrichemicals, and seeds) annually based on their total planted area and received a payout at the end of the season in line with the quantity and quality of tobacco produced. Major investments required by the company included infrastructure for growing tobacco seedlings, tractors for shaping tobacco terraces in the steep hillsides, and drying ovens for the harvested tobacco. Agroecological farmers, on the other hand, said they were able to lower input costs even as they increased production by utilizing more resources internal to the farm, such as reusing waste streams (e.g., composted household food scraps and cow manure) as fertilizer, and relying on ecological pest management, as one agroecological farmer describes below.

> Our agricultural expenses fell by 90% because we don't use anything defensive [against pests], and from there we kept adapting based on the merits of the farm. We take advantage of manure to make the compost, using the leftovers that would have been garbage--that's fertilizer, right?
> Even four years ago we thought we would have to apply some treatments to the grapes because there was a lot of mold. Then while we were still thinking over the situation, our neighbor bought the fungicide, the treatment to apply to

> the grapes, and he produced even less than us that harvest. We just pruned and mowed [under the grapes] and we got a good harvest.

An agroecological banana and palm farmer concurred with this perspective, stating that relative to tobacco, “income isn’t very high, but expenses are low. We don’t have to make all those investments in fertilizer and ‘agrotoxics’.”

In contrast to prior studies that have assessed profitability on organic and diversified farms (Iles and Marsh, 2012; Crowder and Reganold, 2015), agroecological farmers in our case study rarely cited price premiums as a main incentive for transitioning or a critical source of income. In fact, agroecological farmer 13 explicitly stated that it was increased marketed crop diversity, and not price premiums for certified agroecological products, that led to his profits, explaining,

> It’s not the increased price of organics, it’s the diversity. Because money comes in every week, which is an advantage. If you have diversity, you make money every week; if you have a monoculture, just one crop, you make money just in one harvest.

Farmers regularly discussed livelihood diversification through agritourism and increased market access and stability through their networks in Rede Ecovida as important factors in their socioeconomic transition. Through monthly group meetings, learning exchanges, and resource sharing for economic diversification, the Rede Ecovida farmer network embodies many agroecological principles that support transitions, including co-creation of knowledge, social values and diverse diets, participation, fairness, and sustainable land and natural resource governance (Rede Ecovida de Agroecologia, 2004; Wezel et al., 2020). Pluriactivity, a form of rural livelihood diversification in which farmers have both on- and off-farm sources of income (van der Ploeg, 2008), supported both transitioning and agroecological farmers in the case study. As agroecological farmer 11 from Santa Rosa de Lima, who had the smallest cropped area in the study (3 ha), stated,

> It’s also worth saying that tourism is super important for us to be here [on the farm]. If it weren’t for tourism, our small-scale production definitely wouldn’t maintain us. That valorization of seeing, having people coming and recognizing that it is important for us to stay here and continue the work [keeps us going].

Diversifying income sources through pluriactivity is an important mechanism for rural people worldwide to stay on the farm while also building other skills (van der Ploeg, 2008; Schneider and Niederle, 2010). By supporting farms’ financial independence, pluriactivity may also support agroecological transitions and rural transformation (Meek, 2014).

Access to diverse markets

Economic diversification is a fundamental principle of agroecology (Wezel et al., 2020). While conventional farmers earned 59% of their gross annual agricultural income on average from their primary crop, illustrating their high degree of single-market dependence, transitioning and established agroecological farmers earned only a third of their annual agricultural incomes from their highest-earning crop (mean=33% for both). Many farmers in our sample began their transitions by selling smaller volumes of horticultural crops for school lunches in their municipalities through PNAE (n=8). In Brazil, PNAE provides a mediated market for agroecological produce that is supported by federal law, and it can provide a pathway toward increased farm autonomy and diversification, especially for smaller farms (Wittman and Blesh, 2015; Valencia et al., 2019). However, over time, most established agroecological farms in our sample increased the number of sales outlets as their production expanded.

When beginning their transitions, farmers described attending “seminars, get-togethers, and presentations from groups that already worked in agroecology and were incentivizing it, like CEPAGRO,” the partner organization for this case study, often traveling as far as the state capital of Florianópolis (3-4 hours) to participate. The cluster of institutions and social movements supporting agroecological transitions in the region provides a platform for change, enhancing access to ecological and marketing knowledge through public events and to sales opportunities through hundreds of farmers markets. Farmers in the Rede Ecovida network directly coordinate the transport and supply chains of diverse agroecological products through the tri-state Southern Circuit of Circulation and Commercialization of Foods (Magnanti, 2008). Prior research has also found that diversification can stabilize incomes as farmers develop marketing skills to sell their diverse products (Roest et al., 2018). Both transitioning and agroecological farmers discussed the benefits of growing “a little bit of everything,” which was helpful for both household consumption and stable incomes throughout the year. Farmer 9 explained that, to his household, having a diversified farm meant “more healthy food on the table without spending money.”

Re-establishing direct-to-consumer markets and economic partnerships with other farms may increase profitability for smaller farms seeking to diversify (Roest et al., 2018). Farmers involved in agritourism extolled the high value they were able to acquire from direct sales to consumers, relative to selling to wholesalers or other intermediaries who took a cut of the profit. Through the farmer network Rede Ecovida, several transitioning and agroecological farmers in our study joined efforts to create a multi-farm vegetable basket sold weekly to consumers in the state capital of Florianópolis. One transitioning farmer (7) said that joining Rede Ecovida had opened up a host of new opportunities, such as participating in an international event through the Slow Food Movement, which partners with Rede Ecovida in Santa Catarina. Not only that, but the Rede Ecovida network had validated the quality of her farm products, as she said emphatically,

> Before, I didn’t have the courage to say, ‘My product is worth this much, so pay me this much.’ I didn’t know before how much my things were worth. Now, I have the courage to get to the farmers market and say, ‘my cassava flour is worth R$8’… and I end up making that much.

Although some studies have found that the benefits of community-supported agriculture can be reduced as competition increases and the market becomes saturated (Brown and Miller, 2008; Dumont and Baret, 2017), the market for organic produce continues to grow in southern Brazil (EPAGRI, 2019), offering further opportunities for agroecological farmers to diversify their marketing channels and increase their financial independence. While median net agricultural income (per capita) was slightly lower and more variable on agroecological than on conventional farms, agroecological farms had higher total net incomes per capita due to economic diversification through off-farm work (Figure 2.5). Our novel findings highlight the possibility of higher net household income on established agroecological farms when peer networks and stable markets enable redesign and transformation of agricultural systems.

Working conditions

Working hours

Working conditions are a critical consideration in farmers' minds as they ponder whether and how to undertake agroecological transitions (Dumont and Baret, 2017). We found no evidence of increased annual working hours during or following agroecological transitions in our sample (Figure 2.5). On the contrary, time devoted to agricultural labor was highest on conventional farms, with similar total (on- and off-farm) working hours across transition stages (Table 2.3). However, on a per-hectare basis, agroecological farmers worked nearly 3.5 times as many agricultural labor hours per capita as conventional farmers and earned about $1022 USD (239%) more profit (net agricultural income) per ha. This relationship was primarily driven by smaller cultivated areas of higher-value horticultural crops on agroecological farms. This finding epitomizes increased land productivity, in which cultivated area is reduced and labor is concentrated in smaller areas, maximizing profit on the fewer acres in production while maintaining a higher proportion of natural vegetation on remaining land (Chappell and LaValle, 2011). Transitioning farmers, however, worked on average 25% fewer hours per person per hectare relative to conventional farmers but also made only 50% of the profit per hectare. This result related to higher proportions of low-labor perennial crops such as eucalyptus and banana on transitioning farms, which farmers were still learning how to manage productively.

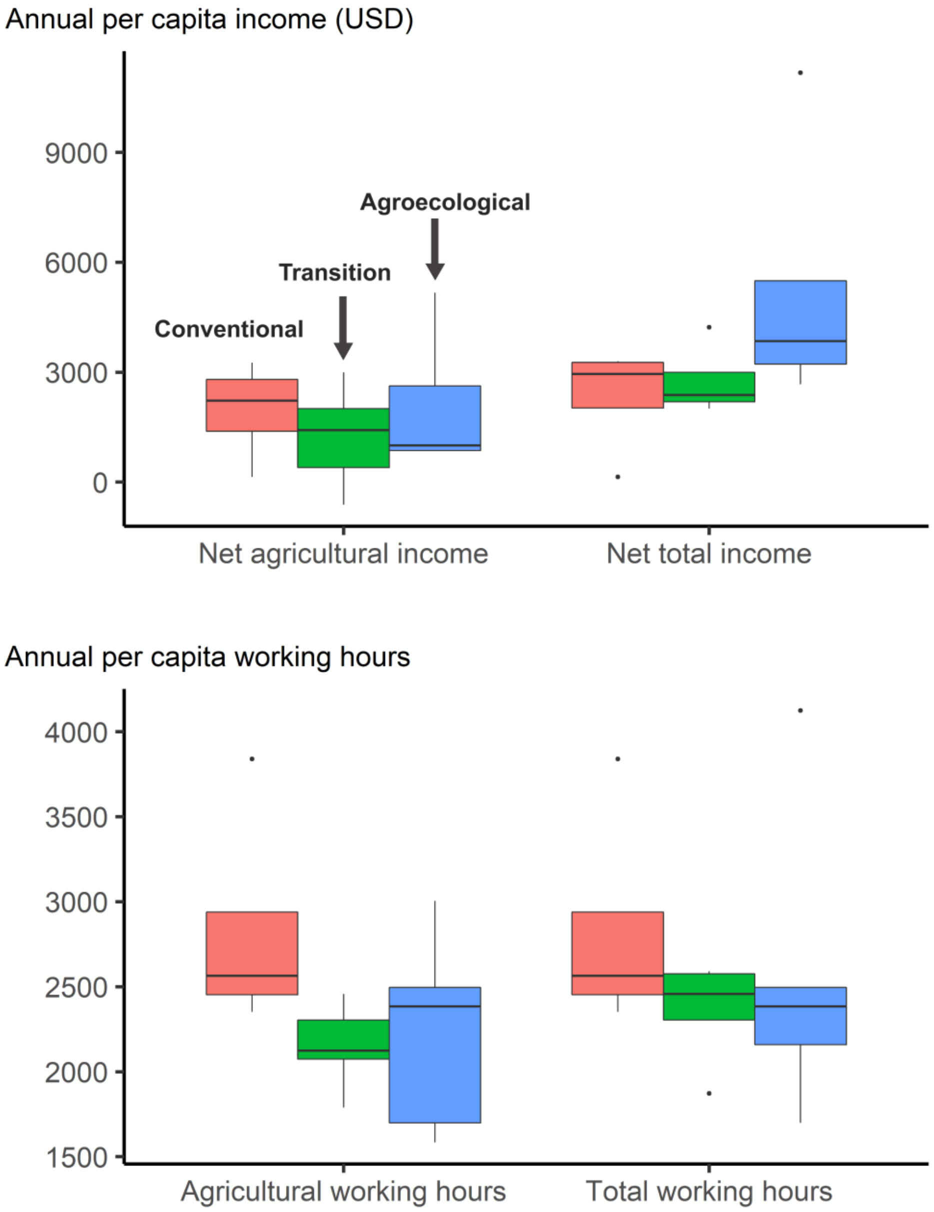

Figure 2.5. Boxplots of farm indicators of financial independence and working conditions at different stages of agroecological transition. Horizontal lines represent median values by transition stage. Two measures for each indicator are shown on the x axes. Financial independence is shown as (1) annual per capita net agricultural income and (2) annual per capita net total income from agriculture and off-farm activities. Working conditions are shown with (1) annual per capita agricultural working hours and (2) annual per capita total working hours from agricultural and off-farm work. Per capita values were calculated as the average annual value for the household divided by the number of household members for income and by the number of workers for labor hours. Net income values represent cash flows and do not include the estimated value of depreciation or household self-provisioning of agricultural products.

Other work comparing agroecological and organic farming systems to conventional systems generally finds increased intensity of agricultural labor in more diversified systems due to fewer possibilities for mechanization and increased complexity in rotations (Galt, 2013; Dumont and Baret, 2017). In our case study, we found the opposite to be true for per-capita working hours, in part due to the labor-intensive nature of tobacco production—from tobacco in the field, to post-harvest drying (using wood-burning stoves), and in the sorting of leaves by quality prior to sale. In addition, both conventional and agroecological farming in eastern Santa Catarina are done with minimal mechanization because of the steepness of fields; fifty percent of farms in the sample owned tractors or rototillers, but farmers used them almost exclusively for tillage, as most crop maintenance and harvesting is done by hand. This case study demonstrates that in the case of tobacco, with mechanization held constant for most tasks, physical work can be more intensive on conventional than on established agroecological farms.

Work quality

Farmers reported that quality of work, or job satisfaction, was an important driver of difference in working conditions across agroecological transitions. Similar to findings from other contexts (Bacon et al., 2012; Dupre et al., 2017), farmers in transition and agroecological farmers both described greater labor and income stability throughout the year due to diversified crop rotations as a benefit to farm operations and worker wellbeing. Farmers also expressed the enjoyment that agroecological farming brought to even a long workday, demonstrating the intrinsic value of their work for their quality of life (Dumont and Baret, 2017). As one farmer (14) with a thriving agritourism business said,

> It's more fun. Sometimes when we are feeling a bit worn out from being out there [in the field] weeding, we come and see a group has made a reservation – every day there is something to look forward to.

Other transitioning and agroecological farmers expressed that their work was better simply because it was "clean, without chemicals," and they could "work in a good mood … with love and with good health."

On the contrary, conventional tobacco farmers framed pesticides as an agricultural tool that drastically reduced the difficulty of their workload and increased their productive capacity. Because tobacco farming in Santa Catarina tends to be labor-limited rather than land-limited, using chemical rather than mechanical weed and pest management can enable farmers to plant more acreage and increase their agricultural income with less physical effort. Conventional farmer 4 described this as a major change over the last several decades, when the state agricultural agency EPAGRI and tobacco companies began promoting chemical pest control in the region, stating:

> Here at our house you won't find any hoes or scythes. Now we have a weed whipper and a chainsaw … Back in the day it was all by hand! We used an ax to cut wood [for the tobacco ovens]. Now we use a chainsaw. In the tobacco, with the pre-emergent herbicides, you don't need to hoe anymore. All of this reduced our labor by 70%.

While herbicides improved the quality of work for this conventional farmer, many agroecological and transitioning farmers emphasized that agrichemical application had severely reduced their quality of life as tobacco growers.

As other studies have theorized, while agroecological farming can be more labor-intensive (which was not the case in our study), farmers often say the work is more meaningful and enables skill-building and self-determination, also appealing to younger generations (Timmermann and Felix, 2015). This concept has also been called "dignified rural livelihoods" in Global South contexts (Blesh and Wittman, 2015) or "active work" in the Global North (Dupre et al., 2017). For small farmers with minimal hired labor who are accustomed to the day-and-night labor of tobacco production, however, agroecological farming, once established, was "about the same" or less work in our case study (Table 2.3).

Occupational health

In 80% of cases, farmers cited occupational health as a main motivation for transitioning to agroecological production in our study. Over the last decade, Brazil has maintained its position as the world’s top importer and third largest consumer of pesticides (after China and the United States), as well as the top global exporter of tobacco (FAOSTAT, 2019). Tobacco cultivation is known for its high agrochemical use, as well as its propensity to cause moderate-to-severe symptoms due to nicotine poisoning (“green tobacco sickness”) when hand-harvested, as it is throughout Santa Catarina (Frois, 2015). On small-scale, remote farms in the Global South, where there is little promotion of or access to personal protective equipment for pesticide application, agroecological work is perceived as safer by farmers. Transitioning and agroecological farmers alike described how the intensive use of agrochemicals affected their daily lives and health. Transitioning farmers (9) who previously grew conventional tobacco and grapes, said:

> There’s always more to improve… but even just having that one thing that is no longer weighing on us—those “agrotoxics,” messing with them and getting headaches, nausea—it’s already wonderful. Our health is much better.

For these farmers, a desire to free themselves from the chemical-intensive agricultural model of conventional tobacco played a key role in their decision to transition to agroecological management in spite of known transition costs.

Occupational health also incorporates mental wellbeing, which can be strained through risk-taking (Dumont and Baret, 2017). Farmers in the case study discussed the risks and costs associated with transitioning from conventional tobacco to diversified agroecological production, though none of the transitioning or established agroecological farmers spoke of their transitions with regret. By creating a social safety net, farmer-driven agroecology movements such as Rede Ecovida can reduce risk for individual farmers to shift their management practices (Hassanein, 1999; Blesh and Wolf, 2014). Even with support from Rede Ecovida, agroecological and transitioning farmers acknowledged that the transition process can take five or more years, especially when starting with former tobacco land. As one agroecological farmer (12) phrased it,

> I always say that the hardest part is to convert… but if you say, I am just going to convert to grow organic and commercialize, you won’t last very long, you’re

> going back to conventional right away. There can be crises in organics, as you don’t have a guaranteed sale, and if you’re far from the city, like us, you can’t sell products as quickly. Now we’ve moved away from [third-party certified] organic to agroecological, but it’s a slow process. Sometimes I say that we worked five or six years just to get a piece of land that we can grow on, because when we started there wasn’t any life in the soil.

Agroecological farmers in the case study were able to take this risk and overcome the opportunity costs of transitioning in part because they were economically diversified, had off-farm sources of income, or both. On average, these farmers also had higher levels of education, smaller households, and more female-headed households than conventional or transitioning tobacco farms (Table 2.2). Each of these demographic characteristics has potential to influence farm decision-making, and prior work has found synergies between women’s empowerment, control over household expenditures, and management decisions in agroecological transitions (Bezner Kerr et al., 2019).

2.4 Study limitations and future opportunities

Using an integrated social-ecological methodology along a gradient of agroecological transition stages, our study found that diversified agroecological farms can achieve comparable agricultural incomes to conventional tobacco farms, with improved working conditions. Due to the labor-intensive production systems for conventional tobacco in Santa Catarina, differences between agroecological and conventional farming in the case study may be magnified relative to regions with differing cropping systems. Compared to other global regions, agroecological farms in southern Brazil also have a historically beneficial policy environment (Medina et al., 2015), strong institutions, and movements, all of which likely contributed to our findings.

There remains much to learn about how farm management practices relate to socioeconomic outcomes for farms undergoing agroecological transitions. With our cross-sectional approach to quantitative data collection on a relatively small number of farms, we cannot determine causality using statistical methods. Instead, we used a purposive sampling approach across three agroecological transition stages combined with qualitative causal explanation to evaluate transition pathways and processes according to farmers' perspectives and experiences (Maxwell, 2004). This detailed, descriptive approach to understanding causal mechanisms and effects can be used to learn deeply about the motivations for and challenges of transitioning, to generate hypotheses for case studies in other regional contexts, or to contribute explanations for findings from quantitative studies on agroecological transitions with larger sample sizes. To determine the applicability of these findings to other contexts, our transition gradient approach could be used to evaluate the environmental sustainability and economic viability of agroecological transitions in larger samples of farms, and across distinct global regions. Further work is also needed to identify policy mechanisms that can support agroecological transitions in regional contexts where peer-support networks such as participatory certification are not already active, and where markets for agroecological products are nascent.

2.5 Conclusion

Using a case study of former tobacco farms transitioning to agroecological management in southern Brazil, we conducted a novel, integrated assessment of ecological and socioeconomic indicators relevant to agroecology. We took a critical realist approach combining qualitative data from interviews and quantitative data on farm management and land use for causal evaluation (Maxwell, 2004). Our results provide initial evidence that moving from an input substitution paradigm to system transformation during agroecological transitions—using diversification, soil cover, and ecological nutrient and pest management—can lead to positive income and labor outcomes on farms with a strong peer-support network and access to diverse markets. System redesign enabled agroecological farms in our case study to reduce their input costs and field labor, allowing more time for farmers to plan complex crop rotations, develop new value-added products, and diversify their marketing structures.

Established agroecological farms in our sample demonstrated the potential for win-win outcomes for ecological and socioeconomic indicators, including both net household income and working conditions. We also found evidence of increased land productivity on agroecological farms, as diversified farms had higher agricultural profits and working hours on a per-hectare basis relative to their conventional tobacco farming counterparts. This transformation was a lengthy process, however, and there were transition costs beyond the official certification period. Farms in transition (0-5 years certified) struggled to manage ecological complexity across the multiple dimensions of farm management, which led to increased work difficulty and reduced profits relative to both agroecological and conventional farms. Additional support for farmers in this early phase of transition could enable their establishment as agroecological farms with ecological, social, and economic advantages. Overall, our findings showcase the potential for income and labor parity between diversified agroecological and conventional farms when adequate support systems are in place. Our study demonstrates that local innovation, participatory certification through farmer networks, and stable markets can enable transformation of agricultural systems for ecological and social sustainability.

Chapter 3 Assessing Cover Crop and Intercrop Performance Along an Agroecological Transition Gradient

Abstract

Diversifying cropping systems with legumes can support multiple ecosystem functions while maintaining crop yields. Yet the impacts of crop diversity on ecosystem functioning may vary depending on farms' baseline environmental conditions and management histories. We conducted a two-year experiment to assess the effects of two diversification practices—cover cropping and intercropping—on nitrogen (N) cycling and productivity across a farm gradient. The field experiment spanned 14 farms in southern Brazil with different long-term management histories. As predicted, farm soils with longer histories of agroecological management had higher levels of soil organic carbon, potentially mineralizable carbon, and extractable phosphorus, captured by a principal component reflecting "biological soil fertility". In the second year of the experiment, the vetch-oat cover crop doubled N inputs to soil compared to fallows across all farms, and N mineralization following cover crop incorporation was twice as high on agroecological farms as on transitioning or conventional farms. Structural equation modeling revealed that after accounting for variation in background fertility across sites, cover crop mixtures explained a further 67% of the variation in soil nitrogen availability at vegetable planting. Consequently, benefits of diversification practices for soil N cycling were ecologically relevant across farms within the short span of our experiment, with the greatest performance overall on agroecological farms. Low soil pH was the strongest constraint on vetch N fixation and on intercrop yield (Land Equivalent Ratio, LER). Overall, the cucumber-snow pea intercrop overyielded relative to monocrops across farms and years (mean LER=1.19), with stronger overyielding in the second year (mean LER=1.27). Though prior work emphasizes interspecific facilitation between crop species in lower fertility soils, our results suggest that overall benefits to ecosystem functioning will continue to build as farmers adopt legume-based diversification practices during transitions to agroecological management.

3.1 Introduction[3]

Intensified agriculture is a primary contributor to global biodiversity loss (Cardinale et al., 2012), drives declines in soil organic carbon (C) stocks (Foley et al., 2011; IPCC, 2014), and pollutes waterways through nitrogen (N), phosphorus (P), and pesticide runoff and leaching (Vitousek et al., 2009; Stehle and Schulz, 2015). Thus, there is an urgent need to transform global agricultural systems to build soil fertility, reduce external input use, and stem the flow of biodiversity loss to sustain agricultural productivity into the future (HLPE, 2019; Barrett et al., 2020). Substantial experimental evidence demonstrates the promise of diversifying farming systems to address these challenges. By applying ecological knowledge to manage greater crop functional diversity from plot to landscape scales, diversified farms can increase biodiversity and multiple ecosystem functions (Kremen et al., 2012), including crop production (Dainese et al., 2019; Tamburini et al., 2020), nutrient cycling and retention (Drinkwater et al., 1998), soil C storage (King and Blesh, 2018), and control of diseases and pests (Dainese et al., 2019). However, the effects of diversification practices on ecosystem functioning vary based on farms' environmental conditions (e.g., parent material, climate, topography) and management histories in ways we are only beginning to understand.

Diversification practices including cover cropping, intercropping, integrated crop-livestock production, and agroforestry are cornerstones of agroecological management (Kremen and Miles, 2012; Wezel et al., 2014). Diversification practices that integrate multiple plant functional groups, such as N-fixing legumes and N-retaining grasses, can have disproportionately large effects on ecosystem functions such as soil N cycling and crop productivity (Wood et al., 2015; Blesh, 2018). Our experiment tested cover cropping and intercropping across a gradient of vegetable farms in southern Brazil that spanned 0 to 20 years of experience with agroecological management. The farms had differences in soil fertility that reflected these distinct management histories. Cover crops are non-harvested crops grown in rotation with harvested crops, and intercropping is the planting of multiple crop species in close spatial proximity. These traditional

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[3] This chapter will be revised and submitted for publication with co-authors Jucinei José Comin, Ilyas Siddique, Donald R. Zak, Leticia Filipini, Renata R. Lucas, and Jennifer Blesh.

practices can also integrate legumes with other crop functional groups to increase the functional diversity of agroecosystems. Combining functionally diverse crop species over space and time can lead to complementary and facilitative interactions that stimulate higher yields in diverse plantings relative to monocultures—known as 'overyielding' (Vandermeer, 1989; Zhang and Li, 2003; Wood et al., 2015). Complementarity in crop mixtures occurs when species have differential resource use through niche differentiation or resource partitioning in space or time (Hooper, 1998; Loreau and Hector, 2001). Facilitation involves the modification of the biotic or abiotic environment by one or more species in a manner that improves resource uptake and/or yield in another species, thereby contributing to overyielding (Bertness and Callaway, 1994; Brooker et al., 2008; Wendling et al., 2017).

Beyond yield benefits, functionally diverse cover crop mixtures can provide multiple ecosystem functions simultaneously (Finney and Kaye, 2016; Santos et al., 2021). Combining legume and grass cover crops, for example, can supply N through biological N fixation, retain N and other nutrients in biomass, suppress weeds, attract pollinators, and increase soil organic matter content over time (Storkey et al., 2015; Blesh, 2018; King and Blesh, 2018). Like cover crop mixtures, vegetable intercrops can be functionally diverse and include legume crops, such that they maximize the potential benefits from interspecific interactions (Gaba et al., 2015).

Given the robust body of evidence on the potential benefits of diversification (Letourneau et al., 2011; Isbell et al., 2017), it is paramount that we grow the scientific and practical knowledge base for effective management of these systems across variable conditions. For example, benefits of intercropping for crop productivity and nutrient uptake depend not only on species selection and other agronomic considerations (e.g., planting date(s), replacement or additive design, seeding rates and row spacing, etc.) (Glaze-Corcoran et al., 2020), but also on soil and climate conditions (Brooker et al., 2015). The effects of diversification practices on ecological functions can be greater in resource-limited soils (Brooker et al., 2015), where facilitation can be stronger in intercrops, for example (He et al., 2013). However, overall ecosystem functions such as biomass production and N cycling tend to be greatest in more fertile soils with a history of crop diversification (Blesh, 2019).

Above all, the performance of diversification practices during "agroecological transitions" remains poorly characterized. Transitions from low-diversity, high-input conventional systems to high-diversity, low-input agroecological farming (i.e., agroecological transitions) involve a complex suite of social and ecological processes that present substantial challenges for farmers across the globe (Ollivier et al., 2018; HLPE, 2019; Tittonell, 2020). Farms undergoing agroecological transitions may encounter differential outcomes of practices such as cover cropping and intercropping based on their soil conditions and prior management practices. Research has shown, for example, that increasing experience with agroecological management, along with increasing soil fertility, helps to improve yield outcomes on organic farms (Martini et al., 2004), and that winter cover cropping contributes to greater soil N cycling and crop productivity over time (Barel et al., 2018). Understanding the effects of agroecosystem diversification relative to other management practices and background environmental conditions is particularly needed for effective decision-making to safeguard long-term soil productive potential (Balvanera et al., 2014). We integrated these complex interactions between management history, background soil fertility, and outcomes of crop diversification into an *a priori* hypothetical model (Figure 3.1), which we tested across a farm management gradient.

On-farm research is a powerful tool for explaining variation in outcomes of diversification practices across environmental conditions and management legacies, thereby building generalizable and practical ecological understanding (Schipanski and Drinkwater, 2012; Blesh, 2019). While prior research has tested the effects of intercropping and crop rotations (Snapp et al., 2010; Mwila et al., 2021) and cover cropping (Vanek and Drinkwater, 2013; Vanek et al., 2020) on farms in regions susceptible to degradation (e.g., mountainous topography, weathered clay soils), no prior work has evaluated the integrated effects of cover cropping and intercropping on ecosystem functions in these vulnerable environmental conditions. Within a context of farms undergoing agroecological transitions in southern Brazil, our study is the first to experimentally evaluate the combined effects of two cropping system diversification practices (cover crop mixtures, followed by vegetable intercrops) on ecosystem functions across a farm gradient.

We conducted our field experiment on 14 farms, representing three different stages of agroecological transition: conventional (not certified), transitioning (0-5 years certified agroecological), and agroecological (>5 years certified). Along this transition gradient, we empirically tested the performance of two diversification practices that aim to increase soil N supply and crop productivity: grass-legume cover crop mixtures and legume-cucurbit intercrops. We expected that five or more years of prior agroecological management (i.e., increased crop diversity, use of ecological nutrient and pest management practices, permanent soil cover) would contribute to soil fertility on the experimental field on each farm.

Specifically, we asked the following research questions: **(1)** How does management history influence soil fertility on farms at different stages of agroecological transition? And, how does soil fertility status affect **(2)** cover crop N assimilation and N availability for subsequent crops? and **(3)** vegetable yields in intercrop relative to monocrop? We first hypothesized that an agroecological management history would lead to higher soil fertility among farms in the study (**H1;** Figure 3.1). Second, we hypothesized that N supply from fixation and subsequent decomposition of the cover crop mixture would increase soil N availability compared to the fallow across all farms, with highest cover crop biomass production and N availability on agroecological farms (**H2**). Finally, we hypothesized that lower-fertility soils, and lower N inputs from the cover crop, would lead to higher yields from vegetable intercrops relative to monocrops, due to complementary nutrient uptake in both species and greater facilitation from legume N fixation (**H3)**.

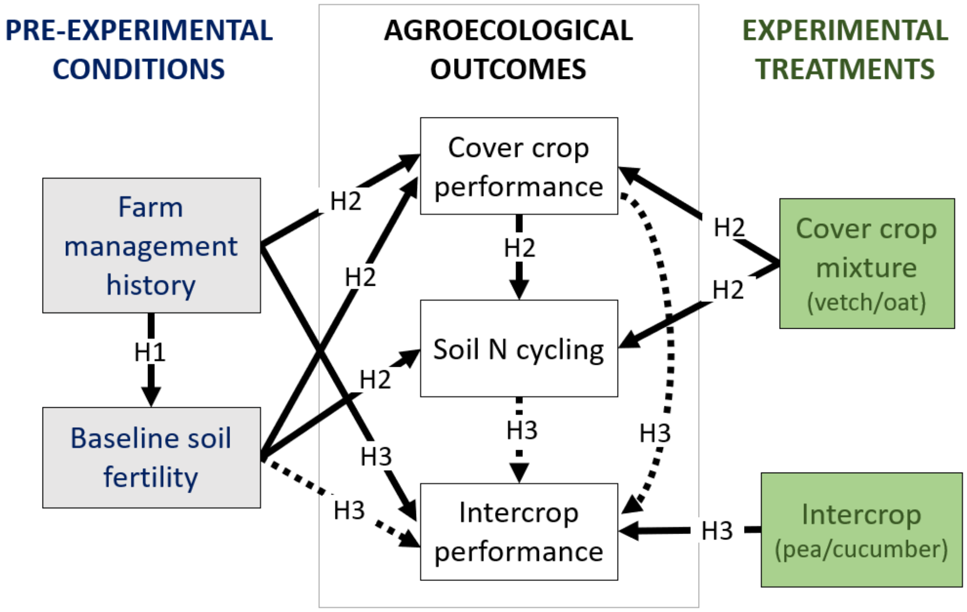


Figure 3.1. Conceptual diagram showing hypothesized relationships between farm management history, baseline soil fertility, cover crop performance, soil N cycling, and intercrop performance. *A priori* hypothesized positive relationships between variables are represented by solid arrows, negative relationships are represented by dotted arrows, and each relationship is labeled with its associated hypothesis. Gray boxes represent pre-experimental variables; green boxes represent experimental treatments; and white boxes represent experimental outcomes.

3.2 Materials and methods

Study site

We conducted our experiment between May 2018 and December 2019 on 14 farms in the eastern coastal highlands region of Santa Catarina, Brazil. The mean altitude of sites was 467 m (+/- 161 m). Eastern Santa Catarina has a subtropical climatic pattern, with mean annual rainfall ranging from 1,500-1,700 mm (Wrege et al., 2012). While 2018 had typical weather patterns for the region, 2019 was a dry year, particularly during the spring months (Table 3.1). All farms were located in the Colonial Serrana Catarinense soil microregion, one of 16 designated microregions in the state of Santa Catarina (EMBRAPA, 2004). Primary soil types in our study site are alic cambisols and red-yellow alic podzols, which tend to be moderately to highly acidic, with limited soil nutrient availability and moisture retention (EMBRAPA, 2004). To support crop production, farmers in the region typically apply lime (calcium hydroxide) to agricultural fields to increase soil pH from <5.5 to 6 (Comissão de Química e Fertilidade do Solo - RS/SC, 2004).

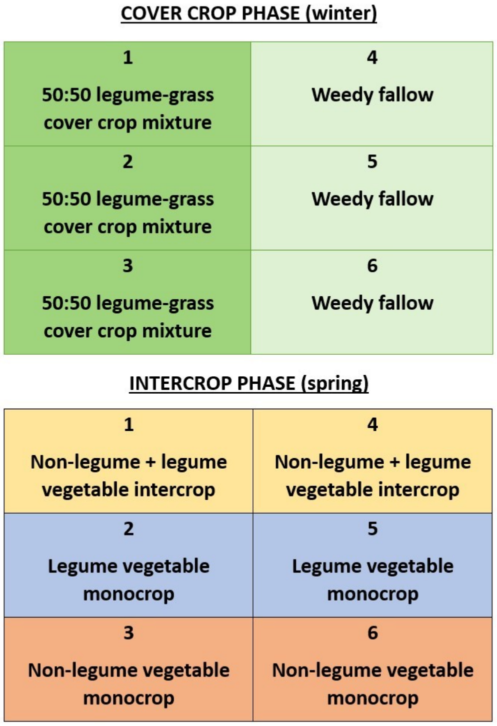
Table 3.1. Climate conditions during the experiment with descriptive statistics. Data represent average conditions across municipalities included in the study (EPAGRI, 2020). Cover crop period: May-August. Intercrop period: September-December.

Year		**Temperature, cover crop period (ºC)**	**Temperature, intercrop period (ºC)**	**Precipitation, cover crop period (mm)**	**Precipitation, intercrop period (mm)**
2018	**Mean**	15	19	354	587
	Median	14	18	348	579
	Std dev.	1	2	12	33
2019	**Mean**	16	19	324	229
	Median	15	18	329	88
	Std dev.	1	1	7	173

Participatory experiment on family farms

Farm selection and the agroecological management index

Partnering with the non-profit Center for the Study and Promotion of Group Agriculture (CEPAGRO, Portuguese acronym), in February 2018 we recruited 15 farms to participate in a 2-year, on-farm experiment (Figure 3.2). Farms were selected based on farmer interest, soil type, and membership status in the Brazilian participatory certification network Rede Ecovida de Agroecologia (Ecovida Network of Agroecology). All farms in the study except one had comparable long-term management histories (e.g., >20 years) as conventional tobacco producers, but we sought farms with distinct recent management histories. Of the 14 farms included in the final dataset, five used conventional practices (high-input, low crop diversity), four were in transition to agroecological certification (0-5 years certified; low-input, moderate crop diversity), and five were experienced agroecological farms (>5 years certified; low-input, high crop diversity). We conducted two detailed interviews on each of these farms to characterize typical management practices. These data were used to calculate four indicators of agroecological management (proportional to field areas): (1) crop and livestock diversity, (2) continuous soil cover (e.g., perennials or cover crops), (3) nutrient management, and (4) pest management. Each of the four management indicators ranged from 0 (least ecological) to 1 (most ecological), and we called the sum of these indicator values the "**agroecological management index**" (Table 3.2). Additional details on calculating the agroecological management index can be found in Appendix 2, and in Chapter 2.



COVER CROP PHASE (winter)

1 50:50 legume-grass cover crop mixture	4 Weedy fallow
2 50:50 legume-grass cover crop mixture	5 Weedy fallow
3 50:50 legume-grass cover crop mixture	6 Weedy fallow

INTERCROP PHASE (spring)

1 Non-legume + legume vegetable intercrop	4 Non-legume + legume vegetable intercrop
2 Legume vegetable monocrop	5 Legume vegetable monocrop
3 Non-legume vegetable monocrop	6 Non-legume vegetable monocrop

Figure 3.2. Experimental design, representing one replicate with six treatments (16 m^2 plot size per treatment) and two factors: winter cover crop treatments, followed by spring intercrop treatments. Scale of inference is the farm field (n=14 farms, one 100 m^2 field per farm).

Experimental design

The fully factorial experiment had six treatments (Figure 3.2): (1) cover crop + pea-cucumber intercrop, (2) cover crop + pea monocrop, (3) cover crop + cucumber monocrop, (4) fallow + pea-cucumber intercrop, (5) fallow + pea monocrop, and (6) fallow + cucurbit monocrop. Due to the timing of farm recruitment, only conventional and transitioning farms participated in the first year of cover cropping (2018); agroecological farms were added to the study during the vegetable intercropping period of 2018 and had their first round of cover cropping in 2019. The cover crop mixture treatment was designed to emulate traditional practices in the region, as well as to include functionally complementary legume and grass species: common vetch (*Vicia sativa* L.) and black oat (*Avena strigosa* Schreb). We also selected vegetables with distinct ecological functional traits, such that intercropping represented an increase in functional diversity relative to monocropped vegetables. Snow peas are N-fixing legumes with a vining, upright structure and a deep rooting pattern, whereas cucumbers are low-lying, non-legume cucurbits that provide groundcover and have a relatively shallow, extensive root system.

Cover crop treatments consisted of two adjacent 50 m^2 plots in each field, one of which was planted with the cover crop mixture (seeding rate: 72 kg/ha black oat and 60 kg/ha common vetch); the other served as a weedy fallow control. Cover crop seeds were inoculated with the Brazilian strain *Rhizobium etli* (SEMIA 384; source: FEPAGRO) at 4 g/kg seed prior to planting. Cover crops were grown until peak flowering, and then cover crops (and weeds in the fallow) were incorporated into the soil by rototiller (n=7 farms) or by hand hoeing (n=7 farms), based on farms' available machinery, between September 5-10 in 2018 and September 10-18 in 2019 (approximately one week following cover crop sampling on each farm).

Vegetables were planted two weeks following cover crop and weed biomass incorporation within a period of 7-10 days across sites. Harvest dates were spaced such that crops were growing for approximately the same period across farms. The 50 m^2 plots were each divided into three intercrop treatments with a ~1 m^2 pathway between each treatment, for a total of 6 treatments randomly assigned to plots per 100 m^2. We planted a climbing variety of snow peas (*Pisum sativum* subsp. *sativum* var. *macrocarpum*, "*Torta de flor roxa*") and pickling cucumber (*Cucumis sativa* L. var. *Pepino HT* 05) in intercrops and in their respective monocrops, using a replacement design (i.e., equivalent crop densities in all treatments). There were five rows of crops per treatment, with only the three middle rows harvested to limit edge effects. In-row spacing was 60 cm for cucumber and 20 cm for peas, with 60 cm between rows in both intercrops and monocrops. Cucumbers were grown as starts for 2.5 weeks before planting, and peas were planted from seed on the same planting date as cucumber starts.

In the summer between January and May 2019 all fields were planted to a sunflower (*Helianthus annuus* L.) crop, which was incorporated into the soil during flowering approximately two weeks prior to cover crop planting in 2019. Because we sought to understand the effects of crop diversification given existing water and nutrient limitations on working farms, the experiment was entirely rainfed and legume N fixation was the sole external N source.

Soil sampling and analysis

Prior to the first cover cropping period, we collected a composite sample of 15-20 soil cores (2.5 cm diameter, 20 cm depth) on both the cover crop and fallow sides of each experimental field (n=28) for analysis of baseline conditions (see Appendix A2.2 for full details). Briefly, soil was analyzed for pH, macro- and micronutrients, and soil organic matter (SOM) by the Santa Catarina State Agricultural Agency (EPAGRI) in Ituporanga, Santa Catarina, Brazil, using standard protocols (Comissão de Química e Fertilidade do Solo - RS/SC, 2004). pH was measured with a pH-meter both with and without Sikora's buffer, and buffered pH is used throughout this paper (Tecnal TEC-11 MP). Soil organic C and total soil N to 20 cm were determined by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, Michigan, USA). We measured soil texture (% clay, sand, and silt) using a total dispersion method with sodium hexametaphosphate (Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), 1997). Bulk density was estimated from the mass of 10 fresh soil cores per treatment, with subsequent accounting for soil moisture.

We measured C mineralization as a baseline indicator of soil microbial activity and biological soil fertility at the start of the experiment, and N mineralization as a response variable following the second year of cover crop treatments. Specifically, using the baseline soil sample, we conducted a short-term (24-hour) C mineralization assay to determine potentially mineralizable C (PMC), which measures the flux of CO_2 following re-wetting of previously air-dried, sieved soil using a Li-Cor (Hurisso et al., 2016). To measure potentially mineralizable N (PMN), we conducted a two-week aerobic incubation using fresh soil collected at vegetable crop planting in the second year of the experiment (spring 2019), two weeks after cover crop and weed biomass incorporation (Drinkwater et al., 1996; Appendix A2.2). PMN was calculated as the difference between extractable soil inorganic N (NH_4^+ and NO_3^-) at the start and end of the incubation. Pre-incubation extractable inorganic N concentration (mg/kg) was used as our measure of soil inorganic N availability at vegetable crop planting.

Cover crop sampling and analysis

Cover crop biomass sampling took place from August 28-September 2 in 2018 and September 4-10 in 2019. During peak flowering of both common vetch and black oat, we destructively harvested the aboveground biomass of cover crop mixtures and weedy fallows from two 0.5 x 0.5 m quadrats of each treatment per field. We took care to avoid treatment edges, cut plant material to the soil surface, and separated harvested plant material by species, grouping all weeds together. Aboveground biomass was dried in a forced-air oven at 60 °C for 48 hours. Following grinding in a Wiley mill to 2 mm, % N and C content was determined by dry combustion on an elemental analyzer (Leco, as above). Community-weighted means were calculated for total aboveground biomass C and N in cover crop species and weeds, to determine the overall C and N inputs to soil following incorporation of biomass on each farm. We measured biological N_2 fixation (BNF) in inoculated common vetch from the cover crop phase of the experiment in 2018 and 2019. Vetch N fixation was estimated using the ^{15}N natural abundance method (Shearer and Kohl, 1986; Blesh, 2019), which compares stable N isotope ratios in the legume and reference species (oat monocultures) (Appendix 2, Section A2.3).

Vegetable crop sampling and analysis

To capture the full production period of both cucumber and pea crops, yield was measured in two harvests, which were approximately 14 days apart on each farm. Harvest dates ran from November 16-December 6 in 2018 and November 20-December 4 in 2019. We measured yield by weighing all harvestable fruit from three designated, representative row sections (6 plants on average per row) per crop type per treatment. Rows were sampled from the center of each treatment to reduce edge effects. We calculated yield as total crop production (g) per plant harvested in each row. Mean yield for each crop type was calculated as the average of the three harvested rows per treatment on a per-plant basis and was then aggregated to the plot and hectare level based on experimental planting densities. Total N harvested, or "N yield" (kg/ha), was calculated for all treatments by multiplying the mean percent nitrogen in each vegetable crop by its yield. Using plot-level yield data, we subsequently calculated the relative yield total (Land Equivalent Ratio, LER) for intercrop treatments by farm using the standard equation (Vandermeer, 1989) (Table 3.2). As a relative measure of total crop production per area, when mean LER > 1, intercrops were considered to have "overyielded" compared to their component monocrops. We calculated the LER for N yield (LER_N in kg N/ha) using the same formula.

At the second vegetable harvest, we destructively sampled whole aboveground crop biomass, including residues and remaining fruits, from the designated experimental rows. Following the harvest, a minimum of six representative cucumbers per treatment (from different plants) per farm were washed in deionized water, air-dried, sliced, and the middle sections were combined into a homogenized, composite sample of ~100 g and then dried for one week at 60 ºC. All peas from each treatment's subplot were washed in deionized water, air-dried, de-stemmed, chopped, and each homogenized sample (35-60 g fresh material) was subsequently dried at 60 ºC in a forced-air oven for 48 h to one week, until fully desiccated. Dried vegetable biomass residues were ground using a Wiley mill; vegetable crop samples were ground in a coffee grinder; and all vegetable samples were analyzed for % C and N on a LECO elemental analyzer.

Statistical analyses

Prior to beginning analysis, we followed recommended protocols to clean and detect outliers in our dataset (Zuur et al., 2009). Out of the 15 farms that participated in the two-year experiment, we ultimately chose to remove one (transitioning) farm from our analysis due to significant differences in baseline (pre-treatment) soil conditions between cover crop and fallow treatments. Hence, results from 14 farms are included in this paper.

Q-Q plots, residuals vs. fitted values, and histograms of residuals for all linear mixed models in the SEMs were examined to assure regression assumptions were not violated (Zelterman, 2015). Variables with non-normal, left-skewed error distributions (i.e., cover crop N supply) were log-transformed for analysis. All statistical analyses were performed in R Statistical Computing software, version 3.6.3, "Holding the Windsock" (R Core Team, 2019). ANOVAs were computed using the lme() function in the nlme package (Pinheiro et al., 2020), coupled with Tukey's and Sidak's post-hoc testing with the emmeans package (Lenth, 2020). We used type II sums of squares unless a significant interaction was present, in which case we used type III (Rencher and Christensen, 2012). Statistical significance was assessed at a 95% confidence interval.

Farm management history and baseline soil fertility

To understand how farm management history is associated with soil fertility (**H1**), we first created an agroecological management index based on the management histories of each experimental field (Appendix 2). We then used Principal Components Analysis (PCA) to reduce the dimensionality of measured soil parameters due to multicollinearity among many of the variables. We used mixed effects linear regression to test relationships between the agroecological management index, and two principal components (PC1 and PC2) (Appendix A2.4). PC1 explained 40% of variation in all soil variables and represented physical soil properties that are not changed by management or are indirectly influenced by management. We therefore labeled PC1 a "textural gradient" (from sandy to clayey) (Table 3.2). PC2 explained an additional 26% of the variation, for a total of 65% explained, and included measures of biological and chemical soil fertility that are more directly influenced by agroecological management practices. We therefore labeled PC2 a "biological soil fertility gradient" across farms. "Farm" was included as a random variable in all models, and "year nested in farm" was the random variable structure in the combined 2018 and 2019 models.

Soil fertility and outcomes of diversification

We first examined the effect of agroecological transition stage on cover crop performance using two-way ANOVA with a dataset that excluded fallow plots, since they did not contain cover crops. Given that cover crop performance can vary based on the number of years of use, we also included experimental year as a fixed effect in cover crop models and included farm as a random effect. Using the full dataset (cover crops and weeds in the fallow), we then evaluated the effects of cover cropping, agroecological transition stage, and their interaction on N cycling outcomes in 2019 (PMN, inorganic N availability) using two-way mixed effects ANOVAs with farm as a random effect. We also tested the significance of overyielding in intercrops (mean LER or LER_N greater than 1) using a one-sided student's t-test.

Table 3.2. Definitions of variables used in data-supported Structural Equation Models (SEMs) to represent ecological concepts from the *a priori* conceptual model (Figure 3.1).

Conceptual model variable	**Structural Equation Model variable**	**Definition**	**Units**
Pre-experimental conditions			
Farm management history	Agroecological management index	Integrated measure of prior use of agroecological practices on experimental fields	Score range: 0-4, from least to most agroecological
Baseline soil fertility	Biological soil fertility gradient	Principal Component (PC) axis with high positive loadings for potentially mineralizable C (PMC) (0.66), total organic C (0.47), and plant-available soil P (0.50)	Unitless Principal Component (PC2), a composite variable
	Soil textural gradient (sand-to-clay)	Principal Component (PC) axis with high negative loadings on soil % sand (-0.50) and bulk density (-0.45), and positive loadings on % clay (0.44) and soil moisture (0.40)	Unitless Principal Component (PC1), a composite variable
	Soil pH	Buffered pH of soil	Unitless
Experimental treatments			
Cover crop mixture (vetch/oat)	Cover crop presence	Cover crop or weedy fallow treatment	Binary presence/absence (0 = fallow, or 1 = cover crop)
Intercrop (pea/cucumber)	Intercrop presence	Intercrop or monocrop treatment	Indirectly modeled through intercrop performance (below)
Agroecological outcomes			
Cover crop performance	Vetch N fixation	Total aboveground N fixed by the vetch cover crop	kg N/ha
	Aboveground biomass N supply	Natural log-transformed aboveground biomass N in cover crops and weeds	kg N/ha
Soil N cycling	Potentially mineralizable N (PMN)	Net N mineralized from soil over a 14-day aerobic incubation	mg N/kg dry soil per day
	Soil inorganic N availability	Inorganic N extracted from soil at vegetable crop planting	mg N/kg dry soil
Intercrop performance	Land Equivalent Ratio (LER)	LER = (intercrop pea yield / monocrop pea yield) + (intercrop cucumber yield / monocrop cucumber yield)	Unitless ratio
	Land Equivalent Ratio for N (LER_N)	LER_N = (intercrop pea N yield / monocrop pea N yield) + (intercrop cucumber N yield / monocrop cucumber N yield)	Unitless ratio

To test the effects of both diversification practices across gradients of soil properties (**H2** and **H3**), we analyzed hypothesized relationships using piecewise Structural Equation Modeling (SEM) with linear mixed-effects models (Lefcheck, 2016). We used several measured variables to represent the broad concepts in our *a priori* hypothetical model (Table 3.2, Figure 3.1). SEMs are a statistical tool increasingly used in ecology to simultaneously test the relative strengths of direct and indirect effect cascades between multiple predictor and response variables (Shipley, 2009; Grace et al., 2016). SEMs were analyzed with the piecewiseSEM package (Lefcheck, 2016), following the procedure recommended in Shipley (2009).

We used two SEMs to test our hypotheses. Because soil N mineralization and inorganic N availability were only measured in 2019, we used the 2019 dataset to test the full set of hypotheses in the hypothetical model (Figure 3.1). This "full SEM" contained a complete set of variables with both diversification treatments and controls for the second year of the experiment (2019). The second SEM we tested was the same as the first, except that it included both 2018 and 2019 datasets, and thus it did not include soil N cycling response variables. For each SEM, we began by testing the meta-model representing our hypotheses. For each of the variables (boxes) in our conceptual meta-model (Figure 3.1), we ran multiple versions of the SEM to test which measured variables led to the best model fit based on R^2, Akaike's Information Criterion, and global model p-value (Lefcheck, 2016; Siddique et al., 2021). Upon determining the final set of measured variables to test in the meta-model, we then sequentially eliminated non-significant predictors until only significant predictors remained, producing a final, data-supported SEM (Figure 3.4; Appendix 2, Table A2.3). PC axes were used as composite variables to represent soil biological, chemical, and textural properties in all models. Soil pH was included as a separate predictor because it is an important mediator of overall soil fertility in the region (EMBRAPA, 2004). Vetch N fixation (kg N/ha) was tested separately from the SEMs in a linear mixed effects model that used only cover crop treatment data (excluding weedy fallows, in which we did not measure N fixation), similar to the cover crop ANOVAs described above.

3.3 Results

H1: Agroecological management and soil fertility

Our measure of farm management history was the agroecological management index, which in agroecological fields was more than double that of conventional fields (2.91 v. 1.22, p=0.005; Appendix 2, Figure A2.1). The mean management index on farms in transition was not significantly different from the other stages (2.21, p=0.13 for difference with conventional and p=0.26 for agroecological). Using SEM, we found a positive effect of the agroecological management index on the biological soil fertility gradient (PC2, Figure 3.3A; marginal R^2=0.34, t=2.66, p=0.02). There was no relationship between the agroecological management index and the textural gradient (PC1, Figure 3.3B; marginal R^2=0.04, t=0.78, p=0.45) or soil pH (t=0.53, p=0.61), nor were there pH or texture differences across treatments. In the partial SEM (using 2018 and 2019 data), pH was a significant and positive predictor of biological soil fertility (PC2, Figure 3.4).

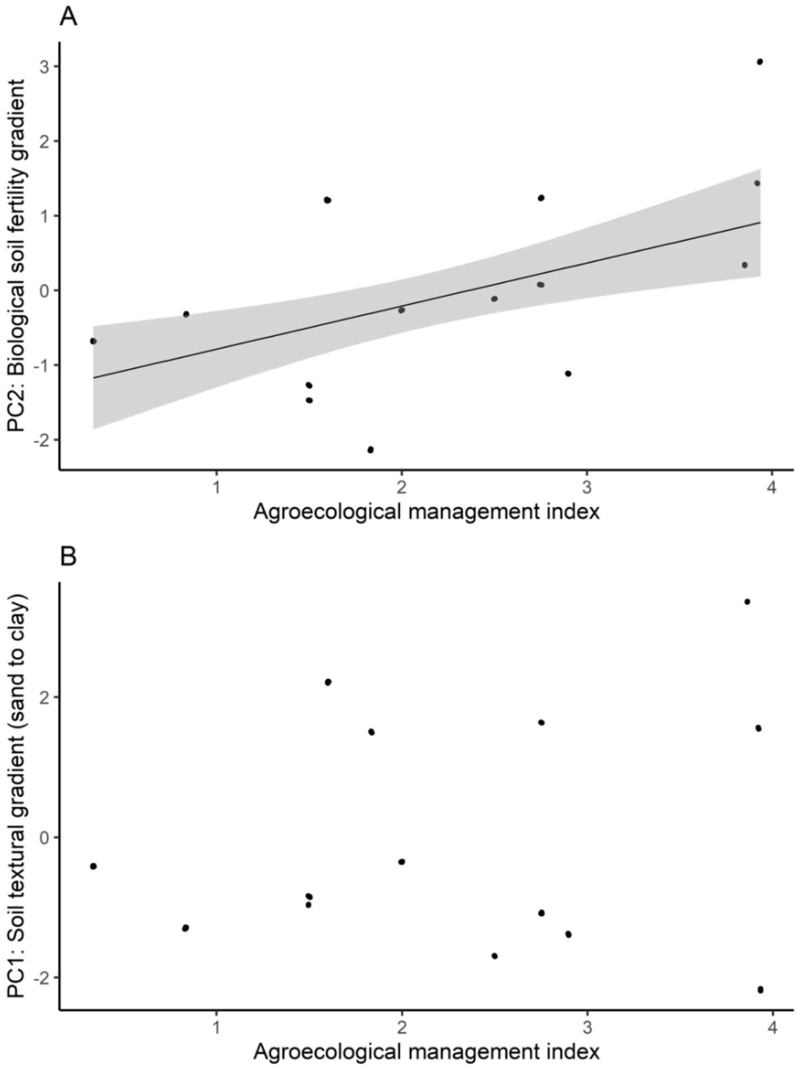


Figure 3.3. Fitted relationships between the agroecological management index for experimental fields and soil biochemical (A) and textural (B) properties. 95% confidence intervals are shown in gray. In the agroecological management index, 0=least ecological, 4=most ecological. See Appendix A2.1 and Chapter 2 for a more detailed description of the index.

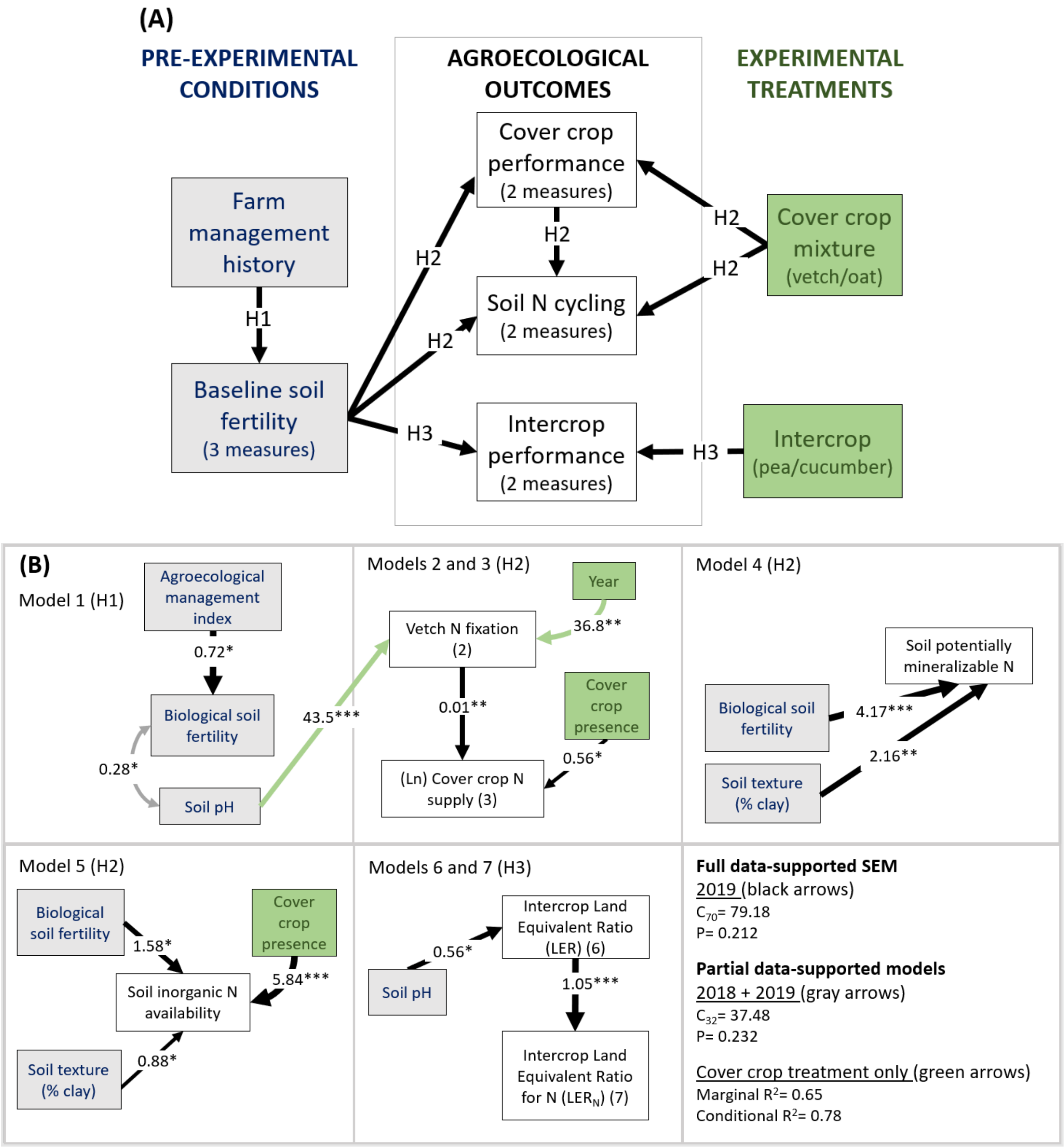


Figure 3.4. Final path diagrams from the Structural Equation Models (SEMs). (A) Broad hypotheses supported by SEMs. (B) The full data-supported SEM links models 1, 3, 4, 5, 6, and 7. Model 2 was run separately from the full SEM, as vetch N fixation was tested using only data from cover crop treatments. The 2018 + 2019 dataset excluded models 4 and 5 and is therefore a partial SEM. Gray boxes represent pre-experimental variables; green boxes represent experimental treatments; and white boxes represent measured outcomes. Solid arrows between boxes indicate positive, directed relationships supported by the data. Double-headed arrows indicate undirected relationships, or correlations. Unstandardized coefficient magnitudes (U) are superimposed on their corresponding arrows, and arrow width represents standard deviation-standardized model coefficients (S). Model statistical significance is connoted by ***$p<0.001$;

**p<0.01; *p<0.05. Intercrop presence is incorporated in the the intercrop Land Equivalent Ratios for yield and N yield (LER and LER_N). Fisher's C statistic, with global degrees of freedom, and global p-values are shown for both SEMs. In SEM, a global p>0.05 indicates a close data-model fit (i.e., no evidence to reject the hypothetical causal model). Complete model results, including standardized and unstandardized coefficients, R^2 values, model fit statistics, and partial regression plots for significant relationships can be found in Appendix A2.5.

H2: Cover crop performance and N cycling across soil gradients

Several measures of baseline soil fertility significantly predicted cover crop N supply from fixation and soil N availability for subsequent crops (Figure 3.4, Appendix 2, Table A2.3). Using a model that included data only from the cover cropped plots (i.e., excluding the weedy fallow), we found that total aboveground N fixed by the vetch in the cover crop mixture was positively related to soil pH (unstandardized regression coefficient, U=35.23, p=0.02) and soil organic carbon (SOC) (U=1.27, p=0.008). After removing one farm with very high SOC (farm 2), which was an outlier, the strong, positive relationship with pH remained (U=43.5, p=0.001) but SOC was no longer a significant predictor (Appendix 2, Figure A2.7). Thus, we present only the pH result in our final model, along with year, which was also a significant fixed effect (Figure 3.4). This effect on aboveground N fixation equates to an increase of nearly 44 kg N/ha with a one unit change in soil pH.

Neither the biological soil fertility gradient (PC2) nor increasing % clay and soil moisture (PC1, the textural gradient) were significant predictors of cover crop N fixation across farms. The proportion of legume biomass in the cover crop mixture (%) was significantly lower in 2018 (mean: 16%, range: 0.5-80%) than 2019 (mean: 39%, range: 4-70%; p=0.026), and vetch N fixation in aboveground biomass (kg/ha) was also significantly lower in 2018 (mean: 26 kg/ha, range: 0.2-71) than in 2019 (mean: 49 kg/ha, range: 4-99, p=0.001) (Figure 3.5). In 2019, with all three transition stages represented, vetch N fixation (kg/ha) tended to be slightly lower on transitioning (mean= 48.0) and agroecological farms (mean =35.1) than on conventional farms (mean = 63.8), but there were no significant differences across transition stages (model p=0.33; Figure 3.5). There was a similar, non-significant trend in N supply from cover crop biomass (Figure 3.5). In the legume, percent N from fixation was consistently high relative to soil-derived N, with a mean of 86% (median: 92%, range: 22-100%) across farms and years (Appendix 2, Figure A2.4). Neither soil gradient explained this variation and there were no significant differences by transition stage. One of the conventional fields was an outlier in the first year of the experiment, with % vetch N from fixation far lower than the mean (22%), but the vetch fixation rate on this site rose to 95% in 2019.

Total cover crop aboveground biomass (vetch and oat) ranged from 1558 to 7916 kg/ha in 2018 and from 2050 to 6704 kg/ha in 2019, with similar means between years (2018: 4169 kg/ha, 2019: 3851 kg/ha). Total biomass was highly correlated with total N supply from cover crops (r=0.85). Across farms, cover crop and weedy fallow treatment biomass did not significantly differ in 2018, due in part to both high weed pressure and poor cover crop performance on some farms, but cover crop biomass was twice that of fallow controls on average in 2019 (Appendix 2, Figure A2.3). Neither soil texture, biological soil fertility, pH, or transition stage were significant predictors of grass-legume mixture biomass, though there was a trend toward lower biomass production on farms in transition, especially in year 1 of the experiment (Figure 3.5). Total aboveground biomass N from the cover crop mixture ranged from 26 to 120 kg N/ha in 2018 and from 40 to 200 kg N/ha in 2019. Cover crop N inputs were driven by vetch N fixation (p=0.007) but were not directly associated with differences in biological soil fertility (p=0.71) or the agroecological management index (p=0.83) (Figure 3.4).

Agroecological farms had 1.7 times higher potentially mineralizable N (PMN) than conventional farms and 2.2 times higher PMN than transitioning farms following cover crop incorporation in 2019 (p=0.003, Figure 3.6). Showing a significant interaction by transition stage in an ANOVA (p=0.001), PMN was 1.6-fold higher in the cover crop treatment than in the fallow controls on conventional farms (p=0.004) but cover cropping had no effect on PMN on transitioning and agroecological farms (Figure 3.6A). In the full SEM, biological soil fertility (PC2; p<0.0001) and increasing soil moisture and clay content (PC1; p=0.002) were strong, positive predictors of PMN (Figure 3.4).

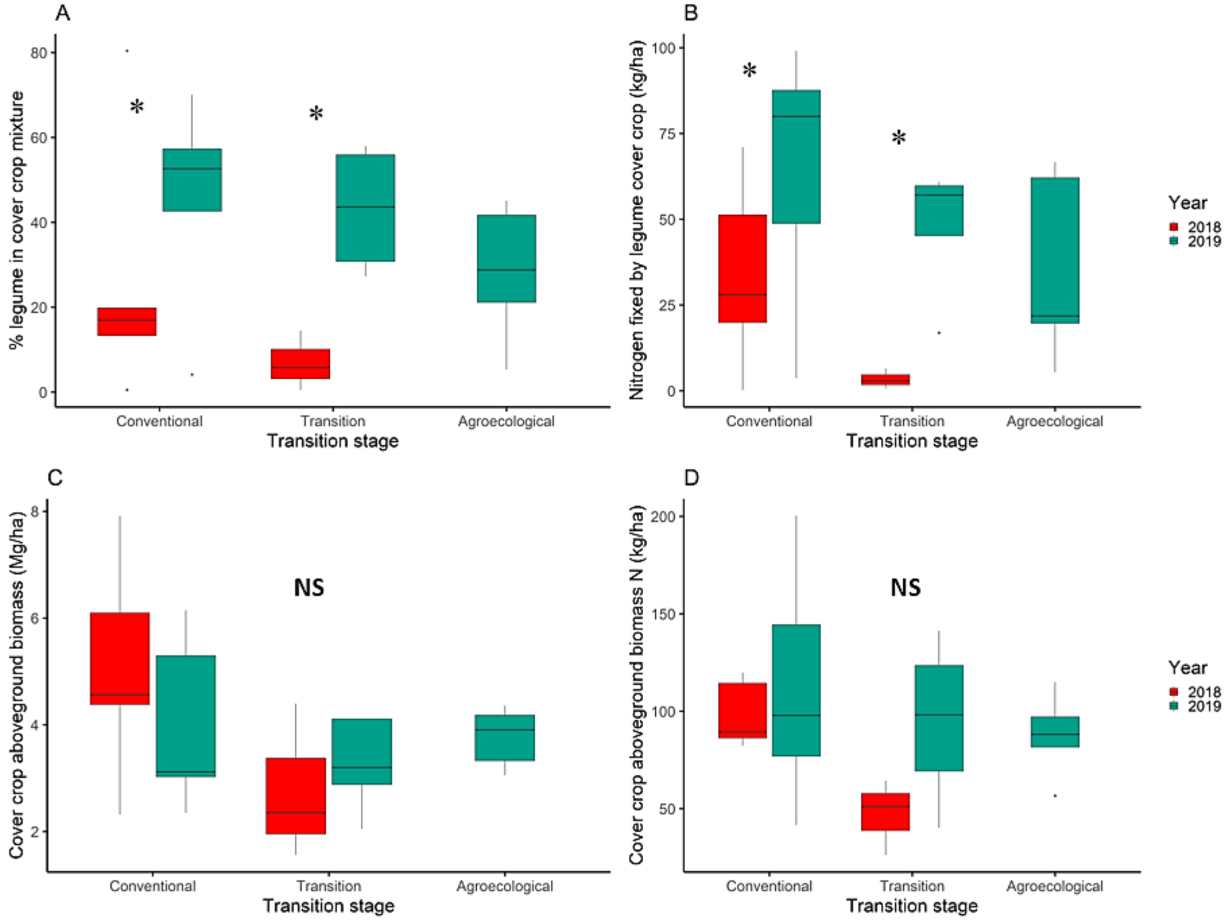


Figure 3.5. Cover crop performance and N inputs across transition stages and years (n=22). A: Proportion of legume (vetch) biomass (%) in cover crop mixtures. B: N fixed by the vetch cover crop in aboveground biomass. C: Total aboveground biomass of cover crop mixtures (including weeds). D: Aboveground biomass N inputs from cover crop mixtures. *indicates significant differences by year (Tukey's, $p<0.05$). NS= no significant differences between transition stages or years.

Positive predictors of soil inorganic N availability included cover crop presence (p=0.0003), biological soil fertility (p=0.008), and higher % clay and soil moisture (p=0.046). Like PMN, soil inorganic N availability at vegetable planting (2 weeks after cover crop incorporation) was highest on agroecological farms (mean: 13.2), followed by farms in transition (mean: 11.4), and then conventional farms (mean: 8.4, p=0.05 for transition stage, Figure 3.6). Soil inorganic N availability was also 1.8 times higher in cover crop treatments than fallows (p=0.0001; Figure 3.6B). On average, there were nearly 6 mg more inorganic N per kg soil available in cover crop treatments than fallows at vegetable crop planting across transition stages ($p<0.0001$).

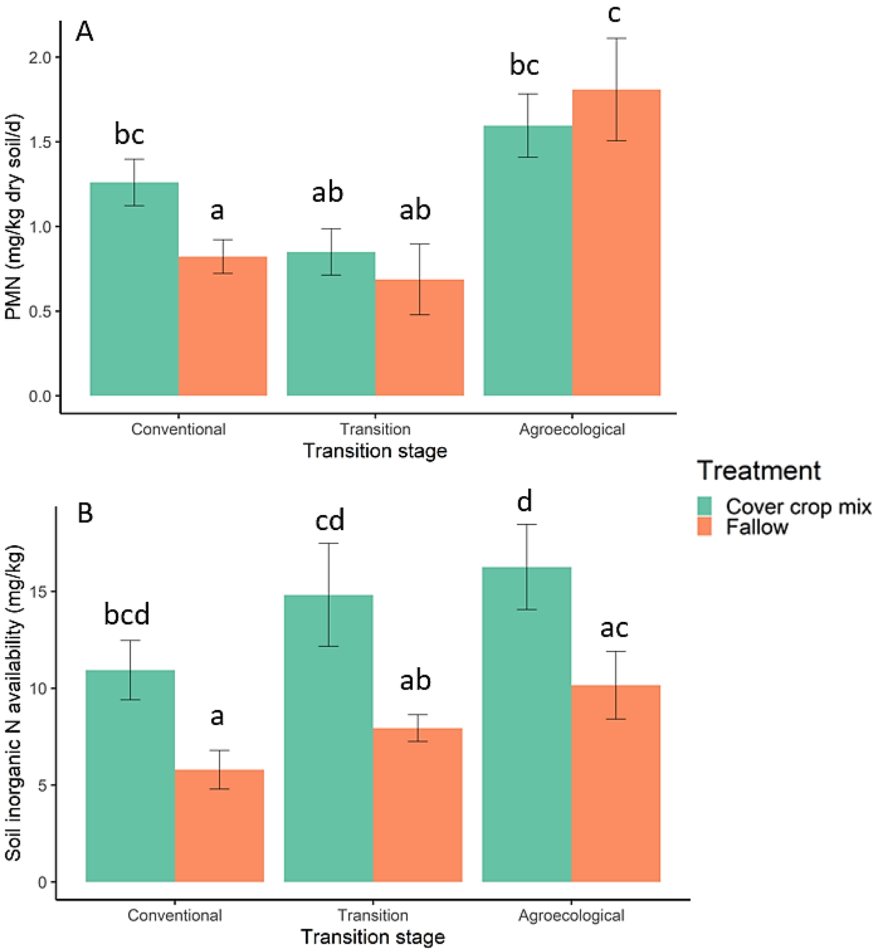


Figure 3.6. Means ± standard error for two measures of soil N cycling, shown by transition stage and cover crop treatment in 2019. A: There was a significant interaction between agroecological transition stage and cover crop treatment on soil potentially mineralizable nitrogen (PMN) following a 14-day aerobic soil incubation. B: Cover crop treatment and agroecological transition stage had an additive effect on soil inorganic N availability at vegetable crop planting. Means that share a letter are not significantly different at a 95% confidence level (Tukey's).

H3: Intercrop performance across soil gradients

Across both years of the experiment, we found that mean LER (1.19, range: 0.18-2.69) was significantly greater than 1 (t=2.09, p=0.021), indicating that intercrops overyielded across farms, a relationship which was driven primarily by slightly higher cucumber yields in intercrops (data not shown). While mean LER_N was 1.08 (range: 0.04-2.95), relative N yields from intercrops were not significantly greater than monocrops across farms and years (t = 0.857, p=

0.198). The Land Equivalent Ratios for yield (LER) and for N yield (LER_N) were highly positively correlated (cor=0.92) (Figure 3.4). Similar to cover crop biomass results, LERs across farms were 28% lower in the first year of the experiment (mean=0.91) than the second (mean=1.27, p=0.035). We found no effects of cover crop treatment on intercrop results.

Similar to the cover crop mixture, soil pH was the only detectable positive driver of vegetable yields (LER) and N yields (LER_N) in intercrop relative to monocrop treatments. We found no direct effect of biological soil fertility or soil texture on intercrop performance. Soil pH had a strong, positive relationship with LER (p=0.024) and thus indirectly drove LER_N, since LER was its main predictor (p<0.001) (Figure 3.4, Appendix 2, Table A2.3). In absolute terms, this equates to an increase of 0.56 of the LER, or a >50% increase in the relative advantage of intercrops for crop yield (LER), with one unit of increase in soil pH. Soil pH had a similar effect on LER_N, but LER was a stronger predictor in SEMs overall.

3.4 Discussion

Biological soil fertility increased across an agroecological transition gradient

Our novel experimental approach across a gradient of farm management history allowed us to identify interactions between soil and management factors that influence the outcomes of legume-based diversification practices. We found that biological soil fertility increased from conventional to agroecological fields, with farms in transition not significantly different from either conventional or agroecological farms (Appendix 2, Figure A2.1). This supports our hypothesis (H1) that soil fertility increases with a history of agroecological management, making it an "agroecological transition gradient" underlying the soil biological fertility gradient. The "transitioning" farms in our study had between 0 and 5 years under agroecological management, relative to more established agroecological farms, which had managed their farms using agroecological practices for 7-20 years (Appendix 2, Table A2.2). The transition group had the lowest prior use of continuous soil cover, and the highest variability in the agroecological management index (Appendix 2, Figure A2.1) and in biological soil fertility relative to conventional or agroecological farms.

Our composite variable of biological soil fertility (PC2), constructed using PCA, represented a linear combination of three biochemical parameters known to relate positively to soil fertility and nutrient availability: SOC (Mg/ha), plant-available P (kg/ha), and potentially mineralizable C (µg CO_2-C/g/d) (Reeves, 1997; Hurisso et al., 2016). Together, PC2 and an orthogonal textural gradient (PC1; from low to high % clay and soil moisture) captured 65% of the variation in soil properties across farms. The biological fertility gradient represented directly "managed" soil characteristics, while properties that are not sensitive to management practices (e.g., parent material, soil age, etc.) fell along the soil textural gradient, and only the former was significantly related to the agroecological management index across farms. It is important to recognize, nonetheless, that biological and physical properties of soils can be influenced by both management and edaphic conditions to varying degrees.

Given that agroecological transitions represent a regime shift involving changes in agroecosystem structure, it is common to see increased variability in ecosystem services (such as crop yields or pest control) for several years before consistent, higher levels of ecosystem functioning are observed (Duru et al., 2015b). Additionally, several outliers in our study reflected unique farm management histories that did not relate directly to their agroecological transition stage. For example, farm 2 in our study had high SOC because the experimental field had been converted from native forest into agricultural land about five years prior to our experiment, driving atypical relationships between SOC and pH relative to other farms with longer agricultural management histories (Appendix 2, Table A2.1, A2.2). Similarly, farm 11 had high plant-available P and K relative to other farms because of long-term application of chicken manure to fields through crop-livestock integration.

More broadly, fields with a history of agroecological management, including high crop and livestock diversity, continuous soil cover, and use of ecological nutrient and pest management practices, had higher biological soil fertility relative to those with simpler crop rotations, reduced soil cover, and reliance on synthetic nutrient and pest management (Figure 3.3). We expected to find this relationship, as a wealth of prior research has identified improvements to soil fertility indicators (e.g., soil organic matter, SOC, and soil microbial activity and biomass) due to individual agroecological management practices, including increased crop rotational diversity

(McDaniel et al., 2014; Tiemann et al., 2015), continuous living plant cover (King and Blesh, 2018), and legume-based nutrient management (Drinkwater et al., 1998).

Perhaps most relevant for our experimental context, a recent study by Teixeira and colleagues (2020) on conventional and agroecological farms in Minas Gerais, Brazil, showed that higher plant diversity mediated the positive effect of agroecological management on soil fertility. Coffee farms across a management gradient in their study also had distinct patterns of nutrient and pest management and soil cover, but these practices were not found to directly affect biological soil fertility after accounting for the effect of increased plant diversity. Distinct from Teixeira et al. (2020), we included the full scope of practices (crop and livestock diversity, continuous soil cover, ecological nutrient and pest management) in our agroecological management index (Appendix 2, Figure A2.1). Using this novel index, we were able to represent the cumulative, integrated effect of agroecological management systems on soil fertility and the performance of diversification practices across a transition gradient. With our structural equation modeling (SEM) analytical approach, we were able to assess the importance of indirect effects of farm management history on cover crop and intercrop performance through long-term changes in soil fertility. SEM also provided unique insight into how distinct components of soil fertility (i.e., pH, biological, and physical attributes) interacted to drive differences in the outcomes of crop diversification practices across the farm gradient.

N supply from cover crops increased with fertility across transition stages

Our findings aligned with the hypothesis that cover crop mixtures would increase soil N cycling at all stages of agroecological transition, but that overall N inputs from residues would be greatest on farms with higher background fertility (H2). In our study, soil inorganic N availability at vegetable planting was nearly two times higher in cover crop treatments than in weedy controls across farms, and it was highest overall on agroecological farms (Figure 3.6). This result could reflect a stronger "priming effect" on higher fertility soils—where the addition of organic inputs stimulates the decomposition of soil organic matter pools (Kuzyakov et al., 2000)—increasing overall soil inorganic N concentrations on agroecological fields compared to those from other transition stages (Blesh and Ying, 2020). The consistent pattern of increased soil inorganic N availability following cover cropping indicates that this diversification practice could be a viable approach to ecological nutrient management during agroecological transitions in southern Brazil and similar regions. As biological soil fertility increased along the transition gradient, we also identified a trend toward reduced legume competitiveness in mixture and lower vetch N fixation (Figure 3.5), as has been shown in prior studies on diversified organic vegetable farms (Blesh, 2019).

Farm management history was an important determinant of soil N mineralization rates and inorganic N availability following cover cropping in our study. One potential mechanism behind the observed patterns of N mineralization and inorganic N availability across the agroecological transition gradient could be higher turnover rates for organic N pools in soils on agroecological and transitioning farms due to increased trophic complexity (Clarholm, 1985; Tiemann et al., 2015), which would lead to greater inorganic N availability following cover crop incorporation. Farms with >5 years of prior agroecological management had 1.6 times higher N availability at vegetable planting than conventional farms, implying that the benefits of incorporating cover crop mixtures into crop rotations as a N source are likely to increase over time. On the other hand, compared to agroecological farms, the 2-fold lower soil N mineralization rate (PMN) on farms in transition also suggests that cover cropping alone may not provision sufficient N during early years of agroecological transitions, necessitating additional nutrient inputs to maintain yields. These findings conform with what prior studies have found on working farms in the US and Europe; recent research has revealed significant increases in SOC and other soil health indicators following 2-5 years of cover cropping (Wood and Bowman, 2021) as well as other agroecological management practices, including conservation tillage and organic soil amendments (Crystal-Ornelas et al., 2021) and addition of perennial forages to grain rotations (King and Blesh, 2018). While we did not measure changes to soil C, our analytical approach across a transition gradient suggests long-term effects of agroecological management for internal N cycling.

The outcomes of farm transitions are influenced by a suite of complex, interacting factors, which made SEM a particularly apt analytical tool to assess the results of our study across an agroecological transition gradient. SEM provides a structured set of procedures to determine the subset of factors that matter most in complex ecological interactions, and to quantify their relative importance. Through SEM, we found that the combined effects of cover crop presence, biological soil fertility (PC2), and clayey soil texture (PC1) best explained soil inorganic N availability at vegetable planting in the second year of our study (Figure 3.4). Furthermore, after accounting for variation in background fertility across sites, cover crop mixtures explained a further 67% of the variation in soil nitrogen availability at vegetable planting. This indicates that the benefits of diversification practices for soil N cycling were ecologically relevant across farms within the short span of our experiment (Figure 3.4; Appendix 2, Table A2.3). After as little as two years, grass-legume cover crop mixtures therefore hold promise to ameliorate conditions of low soil N availability (Figure 3.6). In the first year of our study, however, cover crops on conventional and transitioning farms performed much worse than in the second year, due in part to poor legume establishment in mixtures. Poor legume performance in 2018 could be explained in part by reduced vetch root nodule colonization by the *R.elti* inoculant when first introduced into the experimental fields (Vlassak et al., 1997), though we did not directly assess nodule colonization in our study. In the second year of our study, however, cover crop mixtures increased soil N availability at vegetable planting across farms of all fertility levels and distinct management histories, demonstrating a robust effect across the transition gradient.

Although total soil inorganic N availability was greater on agroecological farms, cover cropping led to the greatest increase in N mineralization rates on conventional farms. Results from a recent laboratory study also suggest that less fertile soils may have stronger increases in measures of microbial activity (C mineralization and enzymatic activity) following new C inputs from cover crop litter (Blesh and Ying, 2020). Recent on-farm work in the United States has identified similar patterns of N mineralization response to cover crop mixtures under laboratory and field conditions using soils from varying fertility levels (Bowles et al., 2014; McDaniel et al., 2016; White et al., 2017), but our study is the first to our knowledge to measure this trend in weathered, subtropical agricultural soils. Targeted future research in other subtropical agricultural contexts could test this hypothesis under field and laboratory conditions, using soils along a fertility or management gradient.

While soil inorganic N availability following cover cropping aligned with our hypothesis (H2), cover crop N inputs from residues did not follow the same pattern (Figure 3.5). Instead, we saw no significant differences in cover crop aboveground biomass or aboveground N assimilation between transition stages. Balanced cover crop biomass production across farm fertility levels could have resulted from the trend toward a higher proportion of legumes in mixture and greater biological N fixation on farms at earlier transition stages than on agroecological farms, reflecting increased mixture complementarity (Figure 3.5A).

Soil pH was the strongest direct (positive) predictor of aboveground N fixation by the legume cover crop across farms (Figure 3.4), and thus indirectly drove N supply by the cover crop mixture, as vetch N fixation was its main predictor. In our study site, where soils are weathered and tend to be moderately to highly acidic (EMBRAPA, 2004), soil pH and the biological soil fertility gradient were positively associated (Figure 3.4). In the case of vetch N fixation, pH was a stronger predictor than biological soil fertility (PC2). This indicates that vetch N fixation may have been constrained in more acidic soils (pH in water < 5.5).

There are two potential rationales for these findings, though we did not measure specific mechanisms: 1) nodulation and N fixation by *Rhizobium etli* in the vetch cover crop were constrained under low soil pH (Graham et al., 1994), and 2) low pH limited uptake of nutrients essential for cover crop growth and vetch N fixation (e.g., P, K, Ca, Fe, Mo) (von Uexküll and Mutert, 1995; Lin et al., 2012). Soil acidity is widely recognized as a constraining factor for symbiotic N fixation (Glenn and Dilworth, 1994; Lin et al., 2012), particularly due to limitations on the ability of *Rhizobia* to survive and persist in soil and to nodulate legumes at low soil pH (Glenn and Dilworth, 1994; Graham et al., 1994). While the species we used to inoculate the vetch in our experiment, *R. etli*, can survive at a pH as low as 5, its growth reaches a maximum between a pH of 5.5 and 7 (Graham et al., 1994; Peick et al., 1999). Prior evidence therefore supports the conclusion that while *R. etli* is moderately acid tolerant, its ability to colonize vetch root nodules may have been inhibited below a soil pH of ~5.5 in our study, also making it less competitive with resident soil rhizobia communities (Vlassak et al., 1997). With regard to the second rationale, earlier research in Santa Catarina found that although Brazilian cover crop varietals are moderately resistant to acidic soils, maximum biomass production for species including black oat and common vetch generally occurs in soils with a pH (in water) between 5.1 and 5.5 (Ernani et al., 2001), equivalent to ~5.5-5.9 pH in buffered solution, which we used in our analysis. Thus, acidic soils likely reduced vetch N fixation and resulting cover crop biomass through a combination of these mechanisms, indicating that cover cropping may be most effective following soil liming in acidic soils with pH < 5.5. Given that an estimated 40% of agricultural soils worldwide are moderately to severely acidic, this finding could be broadly applicable (von Uexküll and Mutert, 1995; Lin et al., 2012).

Studies in more fertile soils have observed the attenuation of legume cover crop N fixation (i.e., % N derived from fixation) where N availability from mineralization is high (Blesh, 2019). In our study, we saw a similar trend toward lower N fixation on agroecological farms relative to other transition stages (Figure 3.5), indicating that fields with lower biological soil fertility may have stimulated greater complementarity between cover crop species relative to the more fertile agroecological soils (except in acidic conditions). This result may also reflect the tendency of legumes to fix more N when mixed with grasses, which they were in our experiment, due to increased competition for soil N in mixture than in a legume monoculture (Wendling et al., 2017; Blesh, 2019). There was one case of only 22% vetch N from fixation on a conventional farm in the first year of our experiment; we attributed this outlier to a legacy effect of conventional nutrient management and potential inorganic N inputs from fields uphill of the experiment in 2018, which could have downregulated legume N fixation (Schipanski and Drinkwater, 2010; Gelfand and Robertson, 2015).

Vegetable intercrops overyielded except in acidic soils with pH < 5.5

Our vegetable intercropping results differed from our hypothesis (H3). Relative yields from intercropping were, on average, greater than monocrops, and this effect did not differ by farm management history or biological soil fertility. Rather, soil acidity was the strongest determinant of relative yields and N yields in intercropping, suggesting that interspecific facilitation was greatest on farms with higher pH soils. Cucumber and snow pea intercrops overyielded relative to monocrops across farms and cover crop treatments (mean LER=1.19) and similarly had consistently higher relative N yields (mean LER_N=1.08). Mean LER was 28% higher in the second year of our study (1.27) than in the first (0.91), which could relate to the drier weather pattern in the 2019 intercrop period. Past grain-legume intercropping studies have identified greater overyielding in intercrops compared to monocrops during periods of low rainfall

(Renwick et al., 2020). Prior studies with functionally diverse vegetable intercrops have also found yield increases (LER=1.17-1.20) in low-input annual cropping systems under experimental station conditions (Franco et al., 2015), but our study is the first to do so along a gradient of working farms with distinct management histories and soil fertility levels.

Mechanisms for overyielding in functionally diverse intercrops—particularly grass-legume intercrops—have been well-studied and include complementarity and facilitation both aboveground and belowground (Hooper, 1998; Brooker et al., 2015; Duchene et al., 2017). It is likely that multiple mechanisms were acting in our experiment to drive overyielding in the snow pea-cucumber intercrop, varying in magnitude based on individual farm conditions. In particular, N fixed by legumes can be transferred to associated species in intercrops (Sakai et al., 2011), and legumes are also known to acidify the rhizosphere, which can solubilize limiting soil nutrients and increase their availability for neighboring plants (Li et al., 2014; Bargaz et al., 2017). Additionally, overyielding can be triggered by plant-pollinator interactions (Brooker et al., 2008) and reduced pest pressure (Gurr et al., 2016), as well as temporal differentiation of resource use (Yu et al., 2015; Engbersen et al., 2021) and biomass accumulation (Dong et al., 2018) related to occupying distinct agroecosystem niches in space and over time. It is therefore probable that a combination of N fixation in the legume (pea) and aboveground and belowground niche partitioning contributed to intercrop overyielding in our study.

Overyielding was robust to distinct soil conditions, except for low soil pH. On farms with acidic soils ($\text{pH} < {\sim}5.5$), intercrop yields (LER) and N yields (LER_N) decreased relative to monocrop yields. We ascribe this result to pH limitations on N fixation and nutrient solubilization, similar to the pattern we saw in the legume cover crops in our experiment. Given that legume N fixation can be limited by both pH and P availability (Bohlool et al., 1992; Ferguson et al., 2013), complementarity and facilitation benefits of diversification practices with legumes may be weaker under acidic, low-fertility soil conditions. Furthermore, there is evidence that legumes' mechanisms to solubilize P and other limiting nutrients, by releasing acid phosphatases in the rhizosphere, are less effective under acidic soil conditions (Li et al., 2010). This relationship is explained by the divergence in dominant forms of P in calcareous (Ca phosphates) and acidic soils (Fe and Al phosphates), the latter of which increase in solubility as pH rises. In intercropping, legumes in calcareous soils can acidify the rooting zone to mobilize P, increasing plant-available P for use in N fixation and facilitating uptake by neighboring intercropped species, which can lead to overyielding (Hinsinger et al., 2011; Bargaz et al., 2017). In acidic soils, however, the increased acidity of legume rooting zones relative to other species in intercrops does not solubilize additional soil nutrients (and could, in fact, inhibit solubilization), thereby limiting the facilitative processes that can lead to yield benefits (Li et al., 2010). It is likely that reduced facilitation between the pea and cucumber in intercrops led to lower LERs on farms with acidic soils in our experiment.

Contrary to our hypothesis (H3), we saw little effect of biological soil fertility (PC2) or farm management history on LER or LER_N. It has been suggested that intercrops have a greater yield advantage under resource limitation, such as under low soil P conditions (Hinsinger et al., 2011), which could have contributed to overyielding in our study site. Intercrops in fields that were on the low end of the biological fertility gradient, but where pH was not a constraint, may have experienced greater benefits of direct facilitation through interspecific interactions that increase nutrient availability in intermingled rooting zones (Hinsinger et al., 2011). This combination of mechanisms may have led to stronger levels of facilitation on low-fertility farms with soil pH > 5.5, relative to farms with higher levels of soil organic C and nutrient availability, resulting in a consistent pattern of overyielding across farms with different fertility levels.

A similar set of mechanisms may explain why we did not observe an effect of cover crop performance on relative yields from intercropping. While we may have expected complementarity between vegetable species to be greater in fallow plots than in cover cropped plots with higher soil N availability, N from cover cropping may not have met vegetable species requirements for N uptake. Total vetch N fixation was notably low across farms (means between 20-50 kg/ha) (Badgley et al., 2007), which aligns with this possibility. In this case, N may have limited crop growth in both cover crop and fallow treatments, driving patterns of facilitation and overyielding in intercrops across the experiment.

Implications for farm management across transitions

Functionally diverse cover crops and intercrops show promise to improve soil nutrient cycling and crop yields in the context of agroecological transitions, especially when species mixtures include legumes (Duchene et al., 2017; Sauer, 2018; Mawois et al., 2019). Yet, variable soil conditions and management histories on farms can affect the extent to which diversification practices enhance ecosystem functions (Schipanski and Drinkwater, 2010; Reiss and Drinkwater, 2020). Grass-legume cover crop mixtures in our study produced more aboveground biomass, supplied more N to subsequent crops, and increased microbial N transformations (relative to fallow) and soil N availability in less acidic and higher fertility soils. The same pattern was true for intercropping; both LER and LER_N significantly increased with pH across the three stages of agroecological transition.

Cover cropping with non-harvested legumes is a practice traditionally used to restore degraded soils in Santa Catarina and elsewhere across the tropics and subtropics (Bohlool et al., 1992; Ernani et al., 2001; Wildner et al., 2004; Stratton et al., 2020). Our results demonstrate that complementary practices to increase soil pH and P availability to crops, such as breeding acid-tolerant legume cultivars or *Rhizobia*, soil liming, and use of rock phosphate and/or composted animal manure, could improve the outcomes of diversification practices such as cover cropping and intercropping with legumes (Ladha and Peoples, 1995; Ferguson et al., 2013). Integrating such practices with legume-based crop diversification would likely lead to greater benefits for soil nutrient cycling and agroecological food production.

While in theory these biophysical challenges to cropping system diversification can be overcome through changes in management (Martini et al., 2004), as described above, in practice farmers undergoing agroecological transitions often face social and economic barriers far beyond the level of the agroecosystem (Bacon et al., 2012). Our study results call into attention the lengthy time horizons of agroecological transitions, as the farms with the highest levels of biological soil fertility and cover crop N supply for vegetable crops in our experiment already had 7-20 years of agroecological experience at the study's start. Although some effects of cover cropping (Blesh, 2019; Wood and Bowman, 2021) and intercropping (this study) on ecosystem functions such as improved soil health and overyielding, respectively, can be observed in a matter of 2-3 years, many farmers lack the supportive socio-political context that enables diversification in the first place. Farmers in our study site in eastern Santa Catarina, Brazil, have multiple layers of external support for their transitions, including access to technical advice through governmental, nonprofit, and educational institutions, a large farmer network (Rede Ecovida) that promotes exchange and shared learning of best practices for agroecological management, and growing markets for agroecological products that facilitate positive socioeconomic outcomes (Valencia et al., 2019; Stratton et al., 2021b). Studies from other regions, on the contrary, demonstrate that these conditions remain uncommon, and farmers can be constrained by access to resources and knowledge, or competing social and economic pressures that prevent adoption of diversification practices (Bacon et al., 2012; Anderson et al., 2019; Mortensen and Smith, 2020). Our research highlights the shifts in soil fertility, farming system diversification, and productivity that amenable policy contexts can enable.

3.5 Conclusions

Through a controlled, two-year experiment on farms across three stages of agroecological transition, we found evidence that biochemical measures of soil fertility (i.e., plant-available P, SOC, and C mineralization rate) were positively associated with agroecological management history. The positive effect of cover cropping on soil inorganic N availability was greater on farms with more fertile soils and longer histories of agroecological management, and N mineralization at vegetable planting was twice as high on agroecological farms as on transitioning or conventional farms. While cover crop mixture biomass did not differ across farms, vetch N fixation tended to be higher on conventional farms than on farms at later stages of transition. We also found evidence of greater complementarity between grass-legume cover crop mixtures and greater N supply to subsequent vegetable crops in less acidic soils. Cucumber and snow pea intercrops consistently overyielded relative to monocrops across farms (mean LER=1.19), with no effect of cover crop treatment, farm management history, or biological soil fertility. As in the cover crop mixture, intercrops had higher relative yields in less acidic soils (pH > 5.5). Above moderate levels of soil pH, our results suggest that the yield advantages of intercropping and complementarity in grass-legume cover crop mixtures may be robust to differences in soil conditions across farms in the subtropics. Further research is needed to identify and test integrated farm management practices that can maximize ecosystem functions from functionally diverse crop mixtures and facilitate agroecological transitions in variable environments.

Chapter 4 Cover Cropping and Intercropping Increase Crop Nutrient Content and Nutrient Yield in Vegetable Agroecosystems

Abstract

Diversified vegetable production shows promise to simultaneously improve agricultural sustainability and provide nutrients for healthy diets. While the nutritional quality of vegetable crops is an important factor for their human health benefits, little research has explored how diversifying cropping systems could affect vegetable nutrient content. In a factorial experiment in southern Brazil, we tested the individual and combined effects of two practices that increase crop functional diversity, grass-legume cover cropping and cucurbit-legume intercropping. We measured the nutrient content (sum of protein and six minerals per 100 g fresh vegetable) and nutrient yield (nutrient content multiplied by crop yield) of two vegetables: snow pea and cucumber. A crop rotation combining both diversification practices increased cucumber yield while maintaining nutrient content, resulting in 5.3 times higher nutrient yield per plant. For the pea crop, coupling cover crop and intercrop treatments led to tradeoffs; peas had 10% lower nutrient yield per plant in the combined treatment but 24-31% higher nutrient yield per plant in individual diversification treatments. Pea total nutrient content increased by 10% under combined diversification treatments compared to the control, with the strongest effects on protein, zinc (Zn), and potassium (K) content. Total nutrient yield per area was highest on average in the cover crop pea treatment, followed by the combined intercrop and cover crop treatment. Results suggest that "biofortification" through diversification can enhance both vegetable yields and nutrient content, with co-benefits for agricultural sustainability.

4.1 Introduction[4]

There is increasing evidence that modern agricultural practices, crop varieties, and changing environmental conditions may be decreasing the nutrient content of our food (Davis, 2009; Myers et al., 2014; Scheelbeek et al., 2018; Alae-Carew et al., 2020). Simultaneously, calls to increase production and consumption of legumes, fruits, and vegetables to support sustainable diets indicate renewed attention on the role of plant-based foods in meeting human nutritional needs while minimizing environmental harm (Willett et al., 2019; Semba et al., 2021). Several lines of research suggest that increasing cropping system diversity can improve yields (Bedoussac et al., 2015; Brooker et al., 2016; Chunjie et al., 2020) and nutrient concentrations (Zhang and Li, 2003; Watson et al., 2012) in staple crop species of nutritional importance. Potential benefits can be magnified in cropping systems that increase crop functional diversity, or the number of crop species with traits that contribute to distinct ecological functions (Martin and Isaac, 2015; Wood et al., 2015). Major gaps in knowledge remain, however; few studies have examined effects of functionally diverse cropping systems on nutrient concentrations and yields of non-staple crops (Franco et al., 2015). As a first attempt to fill this gap, the present study evaluates the individual and combined effects of cover crop and intercrop species mixtures on vegetable crop nutrient content and yields in a field experiment in southern Brazil.

To date, most studies that have assessed the impacts of environmental conditions and management practices on crop nutrient content have focused on input-intensive and monocultural crop production (Davis, 2009; Scheelbeek et al., 2018; Alae-Carew et al., 2020). Inorganic fertilizers have long been known to contribute to a nutrient "dilution effect", whereby crop growth outpaces nutrient uptake following fertilizer additions, resulting in increased yields but reduced concentrations of mineral nutrients in crop tissue (Jarrell and Beverly, 1981). Nutrient dilution is commonly observed in fruit, vegetable, and staple crops under high-input agricultural management (Davis, 2009; Riedell, 2010). In addition to diluted nutrient concentrations, growing fruits and vegetables in input-intensive and low-diversity cropping systems reduces agricultural sustainability, as these systems are associated with numerous

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[4] This chapter will be submitted for publication with co-author Jennifer Blesh following revisions.

externalities and loss of valuable agroecosystem functions such as soil organic carbon (C) storage and nitrogen (N) retention (Tei et al., 2020).

While there have been numerous comparisons of crop nutrients in conventional and organic management systems, with somewhat mixed results (Raigon et al., 2010; Brandt et al., 2011; Hunter et al., 2011; Lester and Saftner, 2011), far fewer studies have evaluated how management practices affect the relationship between crop nutrient content and crop productivity. Moving beyond the broad conventional-organic dichotomy (Shennan et al., 2017), research in sustainable agriculture places increasing emphasis on diversifying farming systems in time and space to enhance multiple, complementary ecosystem functions (Kremen and Miles, 2012; Isbell et al., 2017). For example, adding non-harvested cover crop mixtures in the place of fallows in crop rotations (i.e., increasing temporal functional diversity) can increase soil C storage, nutrient supply, nutrient retention, weed suppression, water infiltration and retention, and productivity over time (Shennan, 2008; Blesh, 2018; Tamburini et al., 2020). Cover crop mixtures that include N-fixing legumes in addition to other functional groups such as grasses and brassicas can better regulate N supply to match crop needs, thereby reducing N losses in the short-term and building soil organic matter in the long-term (Blesh, 2019; Notaris et al., 2021). The few studies that have tested crop nutrient content following specific management practices suggest that incorporating legume cover crops into rotations can increase both N and micronutrient (e.g., zinc (Zn)) uptake in subsequent grain crops (Turmel et al., 2009; Watson et al., 2012). This effect likely derives from legume N fixation and rhizosphere acidification, which can increase the solubility of micronutrients in soil, making them bioavailable to other crops (Watson et al., 2012).

Another common diversification practice, intercropping, increases spatial functional diversity because diverse species interact to provide multiple ecosystem functions at the same time in an agricultural field (Brooker et al., 2015). Intercropping is known to suppress pests and disease and improve crop productivity in both low-input and high-input systems (Brooker et al., 2016; Dainese et al., 2019; Chunjie et al., 2020). Functionally diverse intercrops such as the classic Mesoamerican “three sisters”—combining a legume (bean), grass (maize), and cucurbit (squash)—have greater potential for complementary resource use compared to intercropping three functionally similar species (e.g., three annual grasses) (Vandermeer, 1989; Postma and Lynch, 2012). In the three sisters intercrop, each species occupies different spatial niches, both aboveground and belowground, due in part to their distinct nutrient acquisition traits and root foraging strategies (Zhang et al., 2014; Lopez-Ridaura et al., 2021). With the capacity for symbiotic N fixation, legumes are not N-limited and can also facilitate access to phosphorus (P) and other soil nutrients (Ladha and Peoples, 1995). As they senesce, root and litter biomass inputs from legumes can increase soil nutrient availability for nearby species, facilitating their growth (Vandermeer, 1989). Relative to legumes, grasses are known for deeper root systems, rapid uptake and retention of soil nutrients, and higher biomass accumulation, with high leaf C:N ratios (Wagger et al., 1998). Finally, forbs like cucurbits have shallow, extensive root systems, larger specific leaf areas that can provide soil cover, and lower C:N ratios that reflect greater nutrient demand (Postma and Lynch, 2012; Zhang et al., 2014). When planted together, legumes (bean) fix N, grasses (maize) retain nutrients, and forbs (squash) suppress weeds and maintain soil moisture. The three sisters example reflects broader trends of complementary nutrient use among functionally diverse species in crop mixtures.

Beyond ecological functions, diverse crops can also offer complementary nutritional functions for human consumption (Wood, 2018; Stratton et al., 2020). To assess how production systems meet nutritional needs, recent work has extended the concept of functional diversity from the ecological sciences to include nutritional traits (e.g., concentrations of crop nutrients, crop nutritional composition or quality) (DeClerck et al., 2011; Remans et al., 2011; Wood, 2018). These studies conceptualize nutritional traits such as crop nutrient concentrations as mechanisms that lead to the ecosystem function of providing nutrients for human diets (Wood, 2018). Similar to ecological functional traits (e.g., N fixation), nutritional traits can vary by species and variety (Luckett et al., 2015; Wood, 2018), by management regime (Mitchell et al., 2007; Lester and Saftner, 2011), and across environmental gradients (Wood et al., 2018; Gashu et al., 2021). Given their known effects on ecological functional traits and associated ecosystem functions, agricultural diversification practices such as intercropping and cover cropping are also likely to alter nutritional functional traits of crop species (e.g., Lopez-Ridaura et al., 2021). Both practices have long been shown to increase availability and uptake of certain nutrients such as N and P into aboveground and belowground crop biomass (Hinsinger et al., 2011; Amosse et al., 2014; Li et al., 2014), but the implications of these patterns for the nutrient content and nutrient yield of edible crops destined for human consumption have yet to be studied. Moreover, the combined effects of multiple diversification practices on crop nutritional traits are thus far unexplored.

In this study, we experimentally tested the individual and combined effects of cover cropping and intercropping with functionally diverse species on crop nutrient content and “nutrient yield”, or the total crop nutrients harvested per plant and per area. Our work aims to advance understanding of how crop nutritional traits respond to crop diversification practices intended to increase ecosystem functions, particularly soil nutrient availability. We conducted our experiment with two vegetable species that have distinct ecological and nutritional functional traits: snow pea, a climbing N-fixing legume high in protein, and pickling cucumber, a groundcover cucurbit high in minerals like potassium (K). Our principal research questions were:

(1) What are the individual and combined effects of cover cropping and intercropping on crop nutrient content and nutrient yield, and

(2) How do the effects of these diversification practices on crop nutritional quality vary in species with distinct nutrient acquisition strategies?

First, we hypothesized that a functionally diverse cover crop mixture would increase the supply, retention, and availability of nutrients for the subsequent vegetable crops relative to fallows, thereby increasing crop yields and nutritional quality. Given their distinct N acquisition strategies, we expected cover cropping to increase cucumber N uptake and yields to a greater extent than for pea. Second, we expected a cucumber and snow pea intercrop treatment to increase vegetable nutrient yields and nutrient content relative to monocropped controls, due to niche partitioning and direct facilitation between species through biological N fixation and solubilization of micronutrients. Finally, we expected to find an additive effect of increasing spatial (intercrop) and temporal (cover crop) diversity on vegetable nutrient yields and nutrient content. We anticipated that cucumber would benefit more strongly from the increased availability of N and minerals from cover crop residues and from the legume intercrop, while snow pea would be less competitive in a high-N soil environment following cover cropping.

4.2 Materials and methods

Experimental design and implementation

Our study took place in southern Brazil on the Federal University of Santa Catarina's Ressacada Experimental Farm, in Florianópolis, Santa Catarina, Brazil (27°41'7" S, 48°32'28" W). The site is located in a humid subtropical region with a mean temperature of 21 ± 4ºC and mean annual rainfall of 1415 ± 435 mm. The soil is classified as an Aquic Quartzipsamments (Santos et al., 2021), with a mean pH of 5.6 and a sandy loam texture. Additional baseline soil characteristics by block can be found in Appendix 3 (Table A3.2).

The experiment was active from May to December 2019 and included a cover crop phase (May to early August), followed by an intercrop phase (late August to December) (Appendix 3, Table A3.1, Figure A3.1). The fully factorial experiment had a randomized complete block design in four blocks, with six treatments (3 m x 4 m each) per block and 1 m between each treatment and block (total area=640 m^2, including edge buffers). Treatments included (1) weedy fallow + cucumber monocrop ("control"), (2) cover crop + cucumber monocrop ("cover crop"), (3) weedy fallow + cucumber-pea intercrop ("intercrop"), (4) cover crop + cucumber-pea intercrop (combined diversification; "cover + intercrop"), (5) weedy fallow + pea monocrop ("control"), and (6) cover crop + pea monocrop ("cover crop") (Appendix 3, Table A3.1). In the ten years prior to the experiment, the land was first used to grow high-input vegetable crops from 2009-2012 and was taken out of production from 2013-2018. From August to December 2018, we conducted a pilot intercropping experiment for the present study, after which we left the plot fallow until May 2019. The dominant weed species in the experimental plot was an aggressive perennial nutsedge species *Cyperus rotundus* (L.), which we controlled with manual weeding throughout the vegetable cropping period.

Soil sampling and analysis

We conducted comprehensive soil analysis prior to the experiment, collecting a composite sample of 20 soil cores (2.5 cm diameter, 20 cm depth) per block to measure pH, bulk density, texture, macronutrients (N, K, and P concentrations), micronutrients (calcium (Ca), iron (Fe), magnesium (Mg), Zn, manganese (Mn)), total organic C, and total N. Soil % C and N were measured by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, Michigan, USA). Potentially mineralizable C (PMC) was measured with a Li-Cor as the flux of CO_2 after re-wetting of previously air-dried, sieved soil in a 24-hour C mineralization assay (Hurisso et al., 2016). Inorganic N availability at crop planting was quantified as 2.0 M KCl-extractable NH_4^+ plus NO_3^- and measured colorimetrically using a discrete analyzer (AQ2, Seal Analytical, Mequon, Wisconsin, USA). Soil pH, macronutrients, and micronutrients were analyzed by the Santa Catarina State Agricultural Agency (EPAGRI) in Ituporanga, Santa Catarina, Brazil, using standard protocols (Comissão de Química e Fertilidade do Solo - RS/SC, 2004). More detailed soil analysis methods can be found in Appendix 3, Section A3.1.

Cover crop period

Following two rounds of light tillage (<10 cm) on May 10 and May 13, 2019, cover crop treatments were planted with a grain drill (Campo Nativo SA 11500 A, Vence Tudo, Ibirubá – RS, Brazil) on May 13 in interseeded rows of common vetch (*Vicia sativa* L.) and black oat (*Avena strigosa* Schreb) spaced 10 cm apart. Cover crop mixture seeding rate was 132 kg/ha, divided into 72 kg/ha black oat and 60 kg/ha common vetch. Prior to planting, vetch seeds were inoculated with the Brazilian strain *Rhizobium etli* (SEMIA 384; source: FEPAGRO) at 4 g/kg seed. Cover crops were sampled at peak flowering for both species on September 2-3, 2019, and were then incorporated into the soil using a tractor, with two rounds of light tillage (<10 cm) on September 3 and 10.

During sampling, cover crops and weedy fallow aboveground biomass were destructively harvested from two representative 0.5 x 0.5 m quadrats in each treatment for each block. We cut plant material to the soil surface and avoided treatment edges when sampling. Biomass from both quadrats was combined into a composite sample for each treatment, then separated by species and dried in a forced-air oven at 60 °C for 48 hours, or until fully desiccated. Samples were ground in a Wiley Mill to 2 mm and then analyzed for C and N concentration by dry combustion (as above). To estimate total C and N inputs to soil from aboveground biomass (from cover crop and weedy fallow incorporation), we calculated community-weighted means of aboveground biomass C and N in cover crop species and weeds for each treatment, expressed in kg/ha.

Intercrop period

Vegetable crops were planted 13 days after the second round of tilling for cover crop incorporation, on September 23, 2019. In a replacement intercropping design, which maintains consistent crop densities across treatments, we planted a climbing variety of snow peas (*Pisum sativum* subsp. *sativum* var. *macrocarpum*, "*Torta de flor roxa"*) and pickling cucumber (*Cucumis sativa* L. var. *Pepino HT* 05) in intercrops and in their respective monocrops. There were four rows of crops per treatment (~4 m in length), of which only the two middle rows were harvested to limit edge effects. In-row spacing was 60 cm for cucumber and 20 cm for peas, and between-row spacing was 60 cm for all treatments. Cucumbers (2 plants/plug) were grown as starts for 2.5 weeks before planting on September 23, and peas were planted from seed (2 plants/hole) on the same day. Clear plastic netting was erected to support climbing pea crops one month after planting, on October 23, 2019. All rows of vegetable crops in the experiment were watered using a low-flow drip irrigation system for 6 hours per day to eliminate water limitations on crop growth, from planting until the second harvest. Hand weeding was conducted on a weekly basis throughout the intercropping phase of the experiment.

Vegetable crop sampling and analyses

Vegetable yields

To capture the full production period of both cucumber and pea crops, yield was measured in two harvests, 14 days apart (November 13 and 27). We measured yield by weighing all harvestable fruit from three designated, representative row sections (6 plants on average per section) per crop type per treatment. Rows were sampled from the center of each treatment to reduce edge effects. We calculated yield as total crop production (g) per plant harvested in each row section. After aggregating yields per row over the two harvests, we calculated mean yield for each crop type on a per-plant basis (g/plant) across the three row sections in each treatment. Standard deviation for each crop type per treatment was calculated using mean values per row section. To evaluate total yield per treatment, mean yield per plant for cucumber and pea was then aggregated to the plot and hectare level based on experimental planting densities. In one plot with the combined diversification treatment, the peas and cucumbers failed to establish; this plot was therefore excluded from analysis.

Aboveground biomass of vegetable crops

At the second harvest, we destructively sampled pea and cucumber aboveground biomass (residues, including litter but excluding edible crops) from the designated experimental row sections. Dried aboveground biomass residues were ground using a Wiley mill to 2 mm and analyzed for total C and N on a Leco TruMac CN Analyzer (St. Joseph, MI, USA). Aboveground biomass C and N were calculated in g/plant by crop type per treatment.

Vegetable crop sampling and nutrient analysis

At the first vegetable harvest, when pea production was highest, we separated a portion of the pea yields to use for nutrient analyses. All peas from each treatment's subplot were washed in deionized water, air-dried, de-stemmed, chopped (with a knife washed in deionized water), and each homogenized composite sample (35-60 g fresh material) was subsequently dried at 60 °C in a forced air oven for 48 h to one week, until fully desiccated. Cucumber yields were at a maximum during the second harvest, during which we separated a portion of cucumbers harvested for yield to use in nutrient analyses. Directly following the harvest, a minimum of six representative cucumbers per treatment (from different plants) per treatment were washed in deionized water, air-dried, sliced, and the middle sections were combined into a homogenous composite sample of ~100 g and then dried for one week at 60 °C, until they reached a constant mass.

Pea and cucumber (fruit) samples were finely ground and homogenized using a coffee grinder and analyzed for total C and N (also on the Leco). Remaining sample material was pulverized in a cyclone mill and analyzed for total P, K, calcium (Ca), magnesium (Mg), Zn, and iron (Fe) content. For remaining macro- and micronutrient analyses, we combusted pea and cucumber samples at 550 °C for 12 h in a muffle oven, until only ash and silicaceous material remained. We then digested ashed samples in 1 mL 70% HNO_3 prior to diluting with deionized water to 7% acid and evaluated analytes using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on a Thermo Scientific iCAP Q ICP-MS (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We utilized KED (Kinetic Energy Discrimination) mode on the ICP-MS, which introduces helium gas to the reaction cell chamber to eliminate polyatomic spectral interferences. NIST Apple Leaves Standard Reference Material was used as a quality control standard for all measured nutrients. Using P as an example, nutrient concentrations (% dry matter) were calculated as:

$$\% P = (\mu\mathrm{g\ PO_4} - \mathrm{P\ L^{-1}}) \times (0.010\ L) \times (\frac{1\ \mathrm{g}}{1{,}000{,}000\ \mu\mathrm{g}}) \div (gram\ sample) \times 100\%$$

Percent protein was estimated using established nitrogen-to-protein conversion factors, which were 1 to 4.4 for cucumber and 1 to 5.36 for pea (Mariotti et al., 2008). Percent water for each vegetable crop sample was calculated by weight as the difference in mass before and after drying to a constant weight.

Vegetable nutrient concentration, content, and yield calculations

For the edible portions of cucumber and pea crops we calculated: nutrient concentration (g/g dry weight), nutrient content (g or mg/100 g fresh weight), nutrient yield per plant (mg or g/plant), and nutrient yield per area (kg/ha). We began by calculating nutrient concentrations (g/g dry weight) for each of the measured nutrients: protein, P, K, Mg, Ca, Zn, and Fe. For simplicity of presentation and interpretation, we then created two aggregate measures of nutrient concentrations. The first of these, total mineral concentration, was calculated as the sum of P, K, Mg, Ca, Zn, and Fe concentrations and expressed as an overall percentage of dried sample material by weight. The second aggregate measure was called total nutrient concentration. Total nutrient concentration in each edible crop was calculated as the sum of mineral and protein concentrations (g/g dry weight) for each sample and expressed as % dry weight.

Next, we measured the nutrient content of pea and cucumber crops as grams of nutrient per 100 g fresh weight of each composite vegetable sample. Nutrient content values, unlike nutrient concentrations, incorporated water content within their total mass, as a measure of "fresh weight" that is more representative of potential dietary intakes of vegetables than concentration expressed per dry weight. We calculated aggregate measures of mineral content (P, K, Ca, Mg, Zn, Fe) and total nutrient content (minerals + protein) by summing individual nutrient content values (g/100 g fresh weight) per sample, as above. We express our results for mineral content in mg/100 g given their small values.

Finally, we calculated two measures of nutrient yield, or the total nutrients harvested in edible crops, one on a per-plant basis and another on a per-area basis. On a per-plant basis, nutrient yields were calculated as mean g or mg of nutrients produced per plant over the full harvest season. Individual nutrient yields were calculated by multiplying the mean nutrient concentration (g/g dry weight) in each vegetable crop by its mean yield in dry-matter equivalents (i.e., excluding the weight of water) for a given treatment. Nutrient yields per area were calculated by multiplying nutrient yields per plant by the number of plants per plot (in intercrops this was ½ area of pea, ½ area of cucumber) and then extrapolated to the hectare level. Total nutrient yield per treatment is expressed as kg of nutrients per ha. Similar to the aggregate measures described above, total mineral yield was calculated as the sum of P, K, Ca, Mg, Zn, and Fe nutrient yields, and total nutrient yield was calculated as the sum of mineral yield and protein yield.

Statistical analyses

We used mixed-effects two-way analysis of variance (ANOVA) to analyze our data. We analyzed all data using R Statistical Computing software, version 6.3.0, "Dark and Stormy Night" (R Core Team, 2019). We first tested treatments as the main effects (intercrop, cover crop), an intercrop by cover crop interaction term, and block as a random effect using the lme() function from the R package "nlme" paired with the Anova() function from the "car" package. In cases with no significant interaction, we removed the interaction term and tested the significance of fixed effects using an additive two-way ANOVA model. All model effects were estimated with restricted maximum likelihood (REML) to account for the inclusion of a random variable (experimental block) (Zuur et al., 2009). Using separate ANOVAs by crop type (pea or cucumber), we tested the effects of crop diversification treatments on crop nutrient yield per plant, nutrient content, and nutrient concentration, both for individual nutrients and for aggregate measures. We used a combined dataset including both peas and cucumbers to test the effects of diversification treatments on total mineral, protein, and nutrient yield per area. When we found significant effects in ANOVA models, we completed Tukey's post-hoc testing using the "emmeans" package to assess the significance of differences in treatment means. We used type II sums of squares for additive models and type III sums of squares for interaction models. All models were checked for linear normality by examining histograms of residuals and Q-Q plots, and we checked for heteroscedasticity by plotting graphs of fitted vs. residual values.

4.3 Results

Nutrient yield (g/plant) and nutrient content in cucumber and snow pea

Cucumber

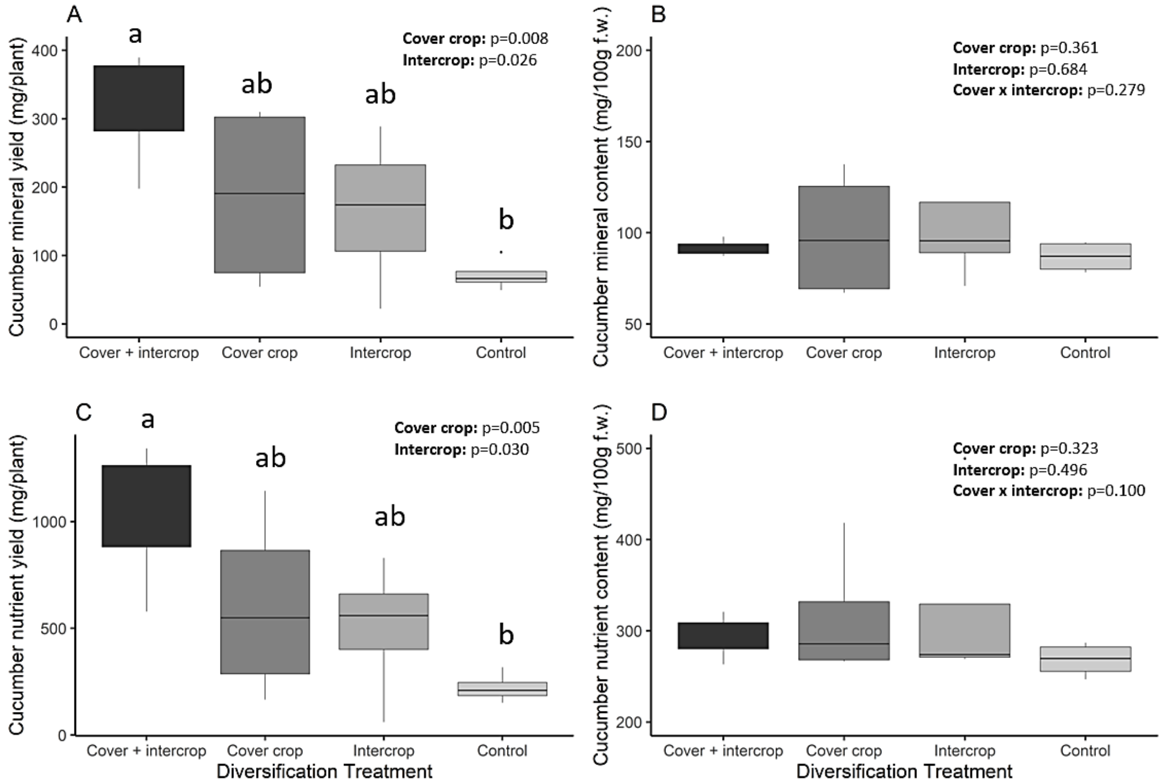


Figure 4.1. Cucumber nutrient yield and nutrient content measured in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control. Minerals included P, K, Ca, Mg, Fe, and Zn. Nutrient yield and content included minerals and protein. Treatments that share a letter are not significantly different at $p<0.05$ (Tukey's). ANOVA model p-values by treatment are shown in top right corner of each panel.

Yield and nutrient yield

Cover cropping and intercropping had combined and individual effects on vegetable yields, mineral content, and total nutrient content, which varied by crop type (Table 4.1). We found a positive, additive effect of cover crop (ANOVA, p=0.003) and intercrop (p=0.02) treatments on cucumber yield (g/plant). On average, cucumbers in the combined cover crop and intercrop diversification treatment yielded 260 g per plant more compared to those in the fallow control. Because crop nutrient content increased proportionally with yield, cucumber mineral yield and total nutrient yield (g/plant) were 5 and 5.3 times higher on average in the combined cover crop and intercrop treatments than in controls (Tukey's $p<0.04$ for both; Figure 4.1A, 4.1C). Cucumber nutrient yields per plant in the individual intercrop and cover crop treatments were 2.75-3 times higher than controls, respectively (Tukey's $p=0.08$). The same pattern was apparent for all individual cucumber nutrient yields on a per-plant basis (Appendix 3, Figure A3.4).

Nutrient content

There were no significant differences in cucumber mineral content or total nutrient content in diversification treatments compared to the control (Figure 4.1). However, cucumbers in individual intercrop and cover crop treatments had slightly higher nutrient content than controls (326 or 314 vs. 268 mg/100g), but this effect diminished in the combined diversification treatment (interaction $p=0.100$, Figure 4.1D). Similar trends were identified for protein and Fe content of cucumbers, which tended to be higher in intercrop and cover crop treatments than in combined diversification or fallow treatments (interaction $p=0.09$ for both) (Appendix 3, Table A3.4, Figure A3.5). Analysis of the C:N ratio of edible cucumber fruit also showed no differences across treatments. Though patterns were similar between cucumber nutrient content (g/100 g fresh weight) and nutrient concentration (g/g dry weight), we identified some differences between the two concentration measures, which we explore for both cucumber and pea crops in Appendix 3, Section A3.3.

Nutrients in non-harvested aboveground biomass

Cucumber aboveground biomass N (excluding the harvested crop) was twice as high in treatments with a cover crop than in control treatments (0.19 vs. 0.09 g N/plant, $p=0.003$), with no difference between intercrops and monocrops. Mean cucumber aboveground biomass C:N was lowest in combined cover crop and intercrop treatments and highest in monocrop fallow plots, showing a trend toward an additive effect of diversification treatments for N uptake into aboveground biomass. Intercropping led to significantly lower C:N of cucumber non-harvested biomass ($p=0.035$).

Table 4.1. Cucumber (A) and snow pea (B) edible crop nutrient composition in 100 g fresh produce, shown as treatment means ± standard deviation (italicized) across four experimental blocks. Total mineral content is the sum of phosphorus (P), potassium (K), magnesium (Mg), Zinc (Zn), iron (Fe), and calcium (Ca) content in mg/100 g. Total nutrient content is the sum of total mineral content and protein content in mg/100 g. N=15 per crop type. Significant treatment effects and interactions are indicated with stars as follows: ***p<0.001, **p<0.01, *p<0.05, ~p<0.10. Additional test statistics are presented in Appendix 3 (Section A3.3).

A					
Cucumber		**Diversification treatment**			
Composition *(in 100 g portion, fresh weight)*	Unit	**Cover + intercrop**	**Cover crop**	**Intercrop**	**Control**
Moisture	%	98.3 *	98.3 *	98.1 *	98.4
	±	*0.15*	*0.44*	*0.58*	*0.14*
Carbon	g	0.73 ~	0.76	0.81	0.69
	±	*0.08*	*0.19*	*0.21*	*0.05*
Nitrogen	g	0.05 ~	0.05	0.05	0.04
	±	*0.01*	*0.01*	*0.01*	*0.00*
Protein	g	0.20 ~	0.22	0.22	0.18
	±	*0.03*	*0.04*	*0.06*	*0.01*
Minerals					
Phosphorus	mg	12.8	13.3	14.6	13.3
	±	*1.0*	*3.6*	*5.9*	*0.6*
Potassium	mg	62.3	69.1	74.2	56.3
	±	*4.5*	*29.8*	*29.1*	*9.2*
Magnesium	mg	7.0	8.6	9.0	7.9
	±	*0.6*	*2.6*	*5.5*	*1.0*
Zinc	mg	0.06	0.07	0.07	0.06
	±	*0.00*	*0.03*	*0.03*	*0.00*
Iron	mg	0.16 ~	0.18	0.21	0.16
	±	*0.01*	*0.05*	*0.09*	*0.01*
Calcium	mg	9.3	7.8	12.0	9.1
	±	*0.9*	*1.5*	*7.2*	*1.3*
Total mineral content	mg	91.7	99.0	110.2	86.8
	±	*5.4*	*35.8*	*47.1*	*8.6*
Total nutrient content	mg	293.7 ~	314.1	326.4	268.2
	±	*28.9*	*71.5*	*108.4*	*18.8*

B					
Snow pea		**Diversification treatment**			
Composition *(in 100 g portion, fresh weight)*	**Unit**	**Cover + intercrop**	**Cover crop**	**Intercrop**	**Control**
Moisture	%	85.6	86.0	85.6	86.3
	±	*0.58*	*0.10*	*0.98*	*0.21*
Carbon	g	6.19	6.03	6.18 ~	5.91
	±	*0.21*	*0.05*	*0.43*	*0.17*
Nitrogen	g	0.45	0.43 ***	0.42 *	0.40
	±	*0.02*	*0.02*	*0.03*	*0.04*
Protein	g	2.39	2.33 ***	2.27 *	2.16
	±	*0.13*	*0.09*	*0.18*	*0.19*
Minerals					
Phosphorus	mg	42.6	57.2	56.6	53.5
	±	*31.7*	*1.5*	*3.0*	*2.7*
Potassium	mg	47.1	42.2	59.4 ~	36.8
	±	*25.5*	*16.3*	*10.2*	*12.6*
Magnesium	mg	32.9	29.7	30.2	31.6
	±	*0.8*	*3.0*	*3.0*	*4.8*
Zinc	mg	0.39	0.33 *	0.30	0.29
	±	*0.01*	*0.03*	*0.04*	*0.11*
Iron	mg	0.55	0.51	0.64	0.57
	±	*0.03*	*0.05*	*0.25*	*0.09*
Calcium	mg	61.8	58.7	60.8	62.8
	±	*2.3*	*2.7*	*5.7*	*3.6*
Total mineral content	mg	185.4	188.6	207.9	185.5
	±	*40.4*	*21.5*	*19.9*	*16.7*
Total nutrient content	mg	2574	2515 **	2478 *	2350
	±	*99.8*	*79.0*	*194.0*	*195.2*

Snow pea

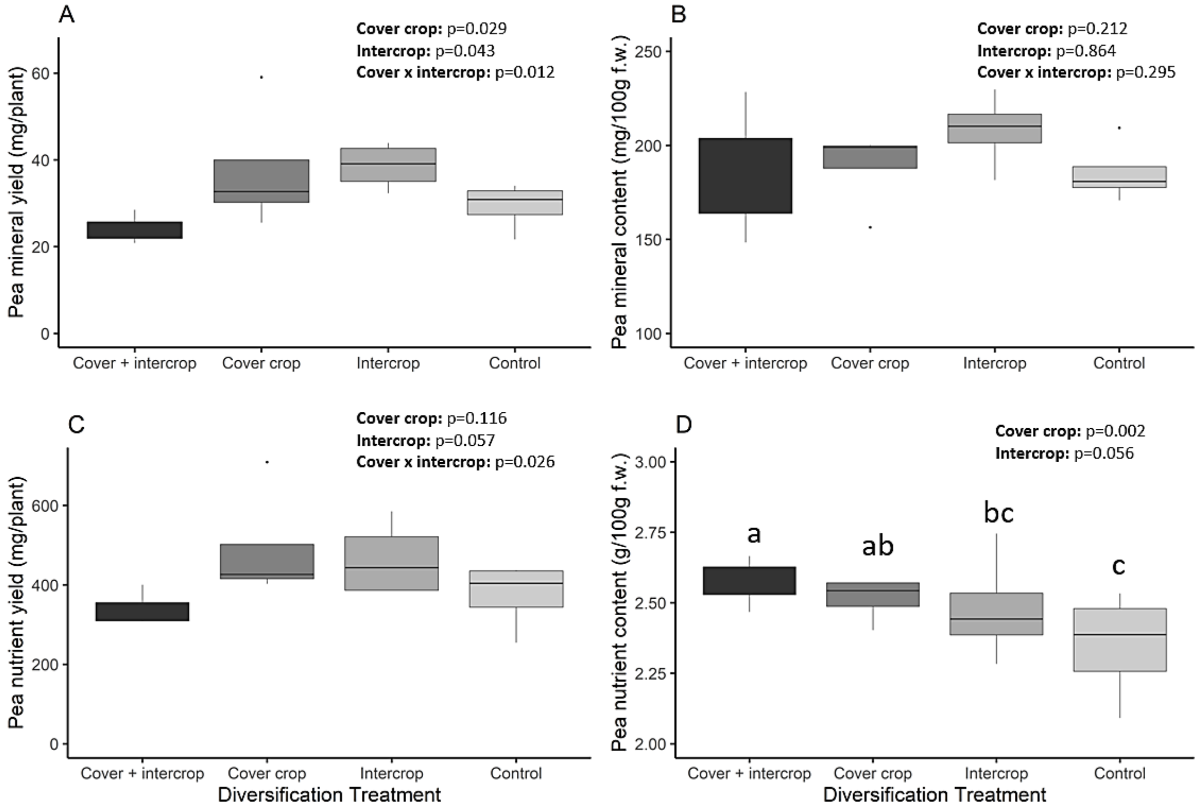


Figure 4.2. Pea nutrient yield and nutrient content measured in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control. Minerals included P, K, Ca, Mg, Fe, and Zn. Nutrient yield and content included minerals and protein. Treatments that share a letter are not significantly different at p<0.05 (Tukey's). ANOVA model p-values by treatment are shown in top right corner of each panel.

Yield and nutrient yield

For snow peas, yields per plant were highest in the cover crop treatment and lowest in the combined diversification treatment (19.7 vs. 13.2 g/plant), with moderate yields in intercrop and control treatments (intercrop: 18.9 g/plant, control: 16.1 g/plant) (ANOVA interaction, p=0.056). These yield differences contributed to the significant cover crop by intercrop interaction on mineral yield per plant (p=0.012) and nutrient yield per plant (p=0.026), though post-hoc pairwise differences were not significant between treatments. Pea mineral yield per plant was 33% higher in intercrops and 28% higher in cover crop treatments than in controls, with similar patterns for nutrient yield per plant, which was 31% higher in cover crop and 24% higher in intercrop treatments than fallow controls (Figure 4.2A, 4.2C). However, we found the opposite pattern in the combined cover crop + intercrop treatment, which had on average 18% lower mineral yield and 10% lower nutrient yield than the control treatment on a per-plant basis. Mean pea nutrient yield per plant was highest in the cover crop treatment, while mineral yield per plant was highest in the intercrop treatment.

Nutrient content

Despite lower pea nutrient yield (g/plant) in combined diversification treatments, cover cropping (p=0.002) and intercropping (p=0.026) had positive, additive effects on total nutrient content in peas (Figure 4.2). Peas in combined diversification treatments had on average 240 mg more nutrients per 100 g fresh weight than peas in the control treatment (Tukey's p=0.026). The main contributor to this effect was protein content (g/100 g fresh weight), which increased by 11% under combined diversification treatments relative to the control (2.41 v. 2.17, Tukey's p=0.017), with significant effects of both cover cropping (p=0.0003) and intercropping (p=0.04). Individual minerals had more variable responses to diversification treatments (Appendix 3, Table A3.5, Figure A3.8), with Zn content increasing by 24% under cover cropping (p=0.02) and K content increasing by 36% under intercropping (p=0.09), but most remaining the same with one or both diversification practices (P, Mg, Fe, Ca). The C:N of snow pea (edible portion) was 13% lower in cover crop treatments than fallows (13.8 vs. 14.7, p=0.004), which was also reflected in the peas' higher protein and total nutrient content under cover crop and combined diversification treatments than in the control.

Nutrients in non-harvested aboveground biomass

In the peas, there were no differences in per-plant aboveground biomass C, N, or C:N (excluding harvested peas) between diversification treatments.

Total protein, mineral, and nutrient yield (kg/ha) by treatment

In addition to per-plant yield effects by crop type, we evaluated the overall nutrient yield for each treatment by area. Total protein yield per area was twice as high on average in treatments that included peas, explained primarily by peas' 10-fold greater protein content relative to cucumbers (Table 4.1). Total mineral yield, however, tended to be highest in the cucumber cover crop treatment on average, which was 2.6 times greater than the cucumber control treatment and 3.2 times greater than the pea control treatment, though these differences were not statistically significant by Tukey's test (p=0.18 and 0.11, respectively) (Figure 4.3). Mean total nutrient yield (kg/ha), which was the sum of total protein and mineral yield for all crops in each treatment, was highest in the combined diversification (27.1 kg/ha) and pea cover crop (31.4 kg/ha) treatments. These treatments achieved total nutrient yields on average 3.8 and 4.4 times higher than the treatment with the lowest total nutrient yield, the cucumber control (7.1 kg/ha) (Tukey's p=0.04 and p=0.006). The intercrop (22.9 kg/ha, Tukey's p=0.09) and pea control treatments (24 kg/ha, Tukey's p=0.07) had marginally higher total nutrient yields than the cucumber control.

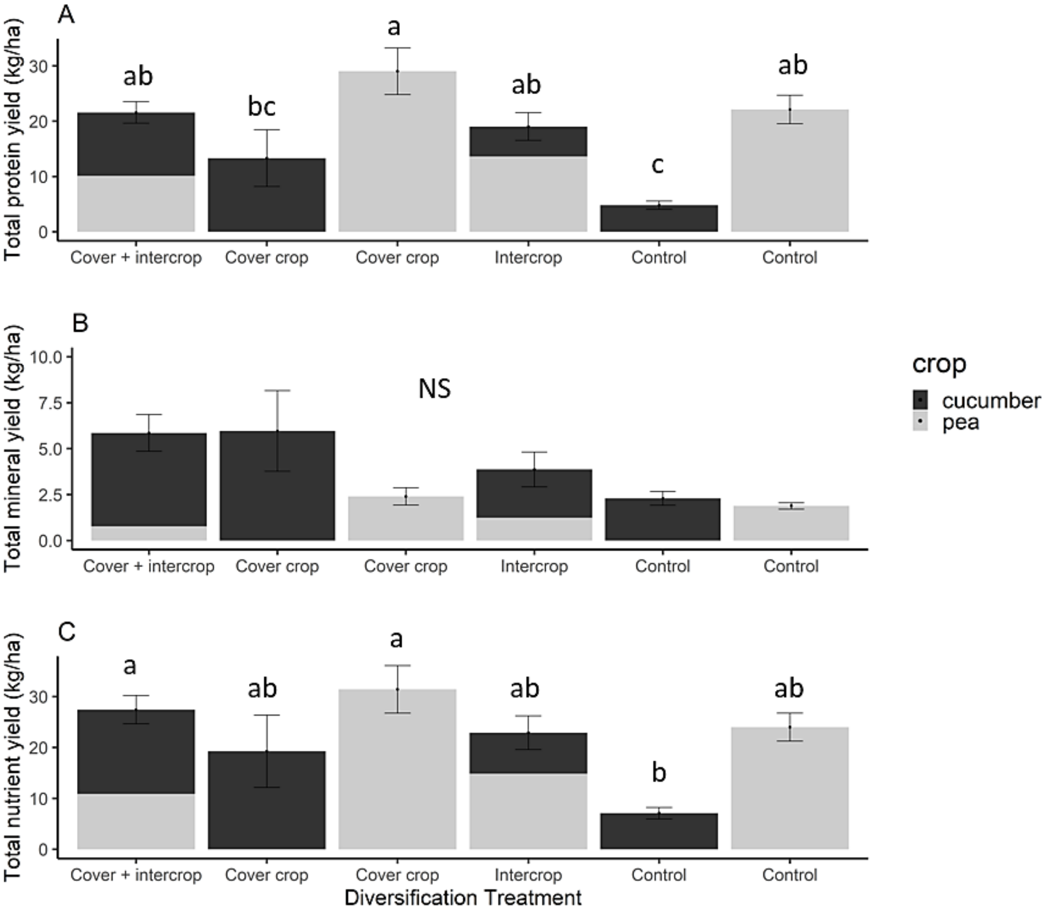


Figure 4.3. Comparing total protein yield, total mineral yield, and total nutrient yield per area by diversification treatment (mean ± standard error). Minerals included P, K, Ca, Mg, Fe, and Zn. Total nutrient yield is the sum of total protein and mineral yield per treatment. In intercropped treatments, we show the contribution of each crop to the total. Treatments that share a letter are not significantly different at $p<0.05$ (Tukey's). NS: no significant treatment effects.

4.4 Discussion

Diversified cropping systems can improve ecosystem functions, but little is known about the effects of diversification on crop nutritional traits, including nutrient yield and nutrient content.

Like plant traits that contribute to ecological functions, nutritional traits are expected to vary with the interaction of management practices (Wood, 2018) and environmental conditions (Wood et al., 2018). Our study found that diversified cropping systems, especially those that leverage crop functional diversity in time and space, can enhance total nutrient yield and increase crop nutrient content compared to less diverse systems. Observed benefits varied by crop species according to its nutrient acquisition strategy.

Cover cropping and intercropping additively increased cucumber nutrient yield while maintaining nutrient content

Our results indicate that using functionally diverse cover cropping and vegetable intercropping can improve cucumber yields without diluting protein or mineral nutrients, including P, K, Fe, Zn, Mg, and Ca (Figure 4.1; Appendix 3, Section A3.3). In other words, cucumber nutrient uptake increased proportionally with yield, resulting in higher total nutrient yield from diversification treatments. We observed this positive outcome for cucumber nutrient yield on both a per-plant and per-area basis (Figure 4.1, Figure 4.3). Cumulatively, this pattern resulted in 5.3 times higher cucumber nutrient yield per plant from combined cover crop and intercrop treatments than from controls. While the magnitude of the cover crop effect was larger than that of intercropping, the combined treatments provided an additional benefit to cucumber yield and nutrient yield per plant. The same pattern was apparent for protein, P, K, Mg, Zn, Ca, and Fe yields in cucumber. Compared to controls, cucumber total nutrient and protein content increased slightly in cover crop and intercrop treatments but not in combined diversification treatments (Appendix 3, Figure A3.5). Similarly, Fe content in cucumbers tended to be 32% higher in the intercrop treatment than the control, but no change was observed in the other diversification treatments. This result differs substantially from prior work testing the effects of inorganic N fertilizer use on crop nutrients, in which an increase in N fertilizer rate often results in a dilution effect for other nutrients (Jarrell and Beverly, 1981; Marles, 2017) unless additional mineral fertilizers are applied (Prasad and Shivay, 2020).

These outcomes for cucumber crops demonstrate that combining cover cropping and intercropping can increase crop uptake of macro- and micronutrients simultaneously. Cover crop mixtures likely increased solubilization, retention, and supply (through N fixation and biomass decomposition) of limiting nutrients (Wendling et al., 2017; Barel et al., 2018; Blesh, 2018), while intercropping led to complementary nutrient uptake and potentially facilitation between cucumber and snow pea (Zuo and Zhang, 2009; Stomph et al., 2020). N was directly supplied to cucumber through decomposition of vetch and oat cover crop residues, while pea sourced its N through fixation. No external sources of mineral nutrients (P, K, Mg, Zn, Fe, Ca), however, were added to the cropping system during the experiment. Rather, mineral nutrient uptake in cucumbers likely increased in proportion to yield due to micronutrient mobilization by both the prior cover crop and intercropped pea roots, particularly via acidification of the rooting zone (Hinsinger et al., 2011; Watson et al., 2012). For this reason, cucumber protein, mineral, and total nutrient yields saw similar gains in individual cover crop and intercrop treatments compared to controls, and there was an additional benefit of combining the two diversification practices (Figure 4.1, Figure 4.3).

Cover cropping and intercropping additively increased pea nutrient content but led to nutrient yield tradeoffs

Snow pea total nutrient content, the sum of protein, P, K, Mg, Zn, Fe, and Ca content, increased by 10% in combined diversification treatments relative to fallow controls and was moderately elevated in the individual cover crop and intercrop treatments (Figure 4.2). The main driver of this effect was protein content, which increased by 11% on average with contributions from both cover crop and intercrop treatments (Appendix 3, Figure A3.8). Two minerals also significantly increased in peas under diversification treatments: mean pea Zn content was highest in combined diversification treatments (1.38 times higher than the control), and pea K content was highest in the intercrop treatment (mean 1.36 times higher than the control).

Similar increases in both protein and mineral content are commonly seen in grass-legume intercropping experiments. A series of 58 field experiments with wheat-pea intercropping across Europe found 13-14% higher protein content on average in intercropped wheat when compared to monoculture, higher and more stable pea and wheat yields, and increased pea N fixation (Bedoussac and Justes, 2010; Bedoussac et al., 2015). Effects were strongest in low-N soils and low-input farms. Past work has also identified higher micronutrient content (56% Zn, 22% Fe, 44% copper) of forage grasses (e.g., perennial ryegrass, festulolium) grown in mixture with legumes compared to monoculture (Watson et al., 2012; Mikronährstoffen et al., 2016). In food crops, studies in China and Turkey have found increased uptake and 1.18-2.82 times higher P, Fe, and Zn content in edible seeds of peanut/maize, chickpea/wheat, and other legume/grass intercrops due to increased nutrient use efficiency and nutrient mobilization (Inal et al., 2007; Zuo and Zhang, 2009; Stomph et al., 2020). To our knowledge, this study is the first to show the combined effects of functionally diverse cover crop and intercrop mixtures on the nutrient content of vegetable crops.

While effects of combined diversification practices were positive for pea protein and mineral content, there were tradeoffs for pea nutrient yields, contrary to our hypothesis. When used separately, cover cropping and intercropping improved pea total nutrient yield per plant by 31% and 24% compared to the control, respectively (Figure 4.2). In the combined cover crop and intercrop treatment, however, pea nutrient yield per plant decreased by 10%, and mineral yield by 18%, compared to the control. This pattern suggests competition or reduced complementarity between intercropped peas and cucumbers in intercrops following cover cropping. Due to this tradeoff, we found no significant differences in total nutrient yield per area between the diversification treatments and monocropped pea control.

Legume species tend to perform best in mixtures under low-N conditions due to their capacity for biological N fixation (Bedoussac et al., 2015); thus, cucumbers could have out-competed pea crops for resources in combined diversification treatments, where soil inorganic N availability was greater (mean=24.8 mg/kg soil) than in the control (mean=14.5 mg/kg, Tukey's p=0.01) at crop planting (Appendix 3, Table A3.3). Because there was high weed pressure in the experimental site, in spite of weekly weeding, peas were also competing with an aggressive perennial nutsedge over the course of the experiment. Another factor that may have played a role in pea yield dynamics in our experiment was that mean vetch N inputs were 1.4 times higher in the combined treatment (mean=122 kg N/ha) than in the cover cropped pea treatment (mean=86 kg N/ha), which likely contributed to lower pea yields in the combined diversification treatment. The lowest total N fixation was in the cover cropped cucumber treatment (59 kg N/ha). These differences in soil N cycling call attention to the variability of ecologically based nutrient management (Reiss and Drinkwater, 2020), as cover crop outcomes diverged under identical cover crop treatment conditions (Appendix 3, Table A3.3). Additional research is needed to identify best practices for cover crop nutrient supply across environmental gradients. Even without the addition of cover cropping, however, intercropping studies sometimes find yield benefits for grasses but tradeoffs in legumes, though this was not the case for intercrop treatments in our study. One previous study identified this pattern for pea intercropped with oat for edible grain production (Mikronährstoffen et al., 2016). Overall, in spite of the reduction in pea nutrient yield per plant (18%) in the combined diversification treatment, the substantial nutrient yield gains per plant in cucumbers (490%, or 5-fold) meant that the overall nutrient production of the combined diversification treatment (kg/ha) was greater than or equal to controls in our study (Figure 4.1, Figure 4.3).

Diversifying cropping systems for agronomic biofortification

Soil Zn and Fe deficiencies are widespread globally and common in Brazil, due to the prevalence of infertile and acidic soils that can inhibit crop nutrient uptake (Alloway, 2008). Such soil deficiencies are associated with human micronutrient deficiencies in populations dependent on local production (Welch, 2002; Barrett and Bevis, 2015; Gashu et al., 2021), contributing to the so-called "hidden hunger" that affects an estimated 2 billion people globally (Gödecke et al., 2018). Given the antagonistic effects of soil micronutrient deficiencies on crop yields, crop nutrient content, and thus on potential human nutrient intakes, there is increasing emphasis on developing strategies to increase the content and bioavailability of minerals like Zn and Fe in soils and edible crops, called biofortification (Khoshgoftarmanesh et al., 2010; Bouis and Saltzman, 2017).

Biofortification typically involves breeding crop cultivars with higher nutrient concentrations than conventional varieties to increase dietary intakes from staple foods (Frossard et al., 2000; Welch and Graham, 2005). This strategy aims to increase the efficiency of crop micronutrient uptake while maintaining productivity (Graham et al., 1999; Khoshgoftarmanesh et al., 2010). Biofortification through breeding relies on multiple methods, including genetic engineering and traditional cross-breeding of higher yielding modern varieties with "heirlooms", wild relatives, and other nutrient-dense varieties (Dwivedi et al., 2019; Ebert, 2020; Erika et al., 2020). In addition to breeding, farm management strategies for biofortification also exist. Agronomic approaches emphasize the application of soil-based or foliar micronutrient fertilizers (Rengel et al., 1999; Khoshgoftarmanesh et al., 2010), but this strategy is input-intensive, expensive, and can be ineffective for increasing nutrient uptake and concentrations in edible crops under heterogeneous soil conditions (Rahman and Schoenau, 2020).

Our study highlights the potential efficacy of another type of agronomic biofortification: biofortification through diversification. Unlike micronutrient fertilization, the targeted use of crop species mixtures with diverse functional traits not only enhances soil nutrient mobility and uptake, but also provides co-benefits for environmental sustainability and resilience of the agricultural system (King and Blesh, 2018; Bowles et al., 2020; Notaris et al., 2021). Approaches like cover cropping and intercropping are comparatively low-cost and adaptable to nearly any environmental conditions, given farmer knowledge of appropriate management and species combinations. The findings of this study show for the first time that integrating multiple diversification practices in space and time could be an effective strategy to increase uptake of low-bioavailability soil nutrients, including K, Fe, and Zn, to improve yields while maintaining or increasing crop nutrient content. The 11% increase in pea protein content we observed with cover cropping and intercropping is also notable, given the urgent need to identify environmentally and socially sustainable protein sources for global populations (Semba et al., 2021). Further research is needed to identify strategies to increase legume protein content while maintaining or improving yields, which was not the case in our study.

Study limitations and future research directions

This experiment was the first to test the combined effects of cover cropping and intercropping on vegetable nutrient content and nutrient yields. While these initial findings are promising, our study had limitations, several of which open opportunities for future research. First and foremost, our study tested a specific combination of cover crop and vegetable species. Cucumber and snow pea were selected for their complementary ecological and nutritional traits, but other vegetable combinations may have benefited more strongly from the preceding cover crop mixture. For example, the positive effects of cover cropping and intercropping on cucumber nutrient yields imply that intercropping complementary non-legume vegetables may have led to greater total nutrient yield gains from the cover crop mixture. Conversely, empirical and modelling studies in Zambia and Malawi have found large productivity gains for both maize and legumes with “doubled-up legumes”, a low-input crop rotation in which two legumes with complementary traits (pigeon pea and peanut/soybean) are intercropped in rotation with maize (Snapp et al., 2010; Smith et al., 2016; Mwila et al., 2021). While these studies did not test the addition of unharvested cover crop mixtures to the rotation, or the mineral yields of the doubled-up legume system, their results could be extended to evaluate these outcomes. For example, future research could assess the effects of semi-perennial legume cover crops and intercrops on crop nutritional traits.

Our study also took place under low-input, irrigated conditions and in a moderately acidic soil. In a high-input system, rainfed conditions, or a calcareous soil, study results would likely differ. Past intercropping research in higher pH soils suggests that the effects of combined diversification practices for soil nutrient mobilization (e.g., Fe, Zn, P) and uptake could be even stronger than what we observed under acidic soil conditions (Zuo and Zhang, 2009; Messaoudi et al., 2020). Under rainfed conditions, interannual variation in cover crop outcomes could be substantial, as cover crop growth, N fixation, and biomass production all depend on water availability. Yet, diverse crop rotations tend to mitigate climate risks in agriculture over time, in both high-input and low-input contexts (Bowles et al., 2020; Williams et al., 2021). So long as there is sufficient rainfall to prevent crop failure, intercropping performs relatively better than monocultures under conditions of water stress or drought (Renwick et al., 2020). In a high-input system where inorganic N and P fertilizers are applied, the effects of cover cropping on crop productivity and protein content would likely be diminished, though cover crops could remain advantageous due to potential micronutrient solubilization and increased bioavailability of Fe, Zn, and Mn for subsequent crops (Wei et al., 2006; Watson et al., 2012). Intercropping typically improves yields in high-input systems due to reduced disease and pest pressure (Chunjie et al., 2020). Thus, the weight of evidence suggests that combining intercropping and cover cropping would benefit crop production under diverse environmental conditions. The impact of combined diversification practices on crop nutrient content has yet to be examined across a range of soils and management systems and merits further study.

4.5 Conclusions

Our study provides initial evidence that combining multiple diversification practices, such as functionally diverse cover crop and intercrop mixtures, can improve nutrient yield and nutrient content in vegetable crops. Specifically, we found that combined diversification practices increased total nutrient yield of cucumber plants, including protein and minerals (P, K, Mg, Zn, Fe, and Ca), with proportional increases in crop nutrient uptake that maintained cucumber nutrient content. In snow pea, mean total nutrient content increased by 10% under cover cropping and intercropping, with even stronger positive effects on protein, Zn, and K content relative to the control. Pea total nutrient yield per plant increased by 24-31% on average under individual cover crop and intercrop treatments compared to controls but decreased by 10% when diversification practices were combined. Future diversification studies could investigate mechanisms behind this tradeoff between legume crop yield and quality. Overall, total nutrient production (kg/ha) was greatest in the combined diversification and cover crop treatments, which were significantly higher than the cucumber but not the pea control. Further research is required to determine the generalizability of results to distinct cropping systems and environmental conditions. Our findings suggest that "biofortification through diversification" could be a viable approach to improve vegetable yields and nutrient content while enhancing environmental sustainability in low-input agricultural systems.

Chapter 5 Conclusion

This dissertation sheds new light on the intersecting social, ecological, and nutritional functions of agroecosystems in transition. As a subset of broader sustainability transitions, agroecological transitions represent a fundamental restructuring of agricultural systems (Gliessman, 2014; Tittonell, 2020), such that changes in management practices, including crop diversification, likely have implications for farm income, working conditions, nutrient cycling, and crop nutrient yield, among other agroecosystem functions. Until now, these multidimensional outcomes of crop diversification have not been evaluated at different stages of agroecological transition. In Chapters 2 and 3 of this dissertation, I employed a unique experimental approach, interviewing farmers and testing two diversification practices, cover cropping and intercropping with functionally diverse species, across a gradient of farms transitioning from conventional tobacco to agroecological vegetable production in southern Brazil. In Chapter 4, I added another dimension to the analysis by studying the crop nutrient outcomes of cover cropping and intercropping on an experimental farm in the same region.

5.1 Summary of findings: crop diversification and agroecosystem functions across transitions

As a whole, there are three main takeaways from this dissertation. First, I found that adding cover crop and intercrop species mixtures to crop rotations had significant, positive effects on both ecological and nutritional agroecosystem functions after just one to two years. Specifically, in Chapter 3, I identified consistently higher soil N availability in rotations with cover crops compared to fallows and greater relative productivity from intercrops than monocrops across farms in the second year of the experiment. Background soil fertility, including pH, biochemical, and textural properties, mediated the effects of diversification practices on the ecological functions of biological N fixation by the vetch cover crop, soil N mineralization, and inorganic N availability, and overyielding in intercrops. After controlling for this environmental variation, cover cropping and intercropping contributed significantly to soil N cycling and vegetable crop productivity, respectively, across the agroecological transition gradient. Complementing these findings, in Chapter 4 I found that total nutrient yields (kg/ha) from diversification treatments were 3.8-4.4 times higher than the cucumber control in a factorial field experiment, though they were not different than the pea control. Additionally, total pea nutrient content (protein and six minerals) was 10% higher following a cover crop mixture, while there was no change in cucumber nutrient content as yield increased. These results suggest that biofortification through diversification could be a viable approach to improve vegetable yields and nutrient content while enhancing environmental sustainability in low-input agroecosystems.

A second major takeaway of this dissertation is that the magnitudes of measured socioeconomic and ecological outcomes were higher on established agroecological farms than on conventional farms in the study region. The average agroecological farm in our experiment raised 12 times as many crop and livestock species (mean=24) as the average conventional farm (mean=2) and used ecological nutrient and pest management strategies on larger proportions of their farms. Driven in part by these management histories, the ecological results from Chapter 3 showed greater average biological soil fertility (a composite variable including soil organic C, C mineralization, and plant-available P) at baseline and higher soil N mineralization and inorganic N availability after cover cropping on agroecological than conventional farms. In parallel, Chapter 2 found that the socioeconomic outcome of total household income (mean, per capita) was over twice as high on agroecological as on conventional farms, and mean weekly labor hours were the same or slightly lower per capita on agroecological (mean: 49) compared to conventional (mean: 54) farms. Qualitative data also underscored improved farmer-reported work quality and occupational health on agroecological relative to conventional farms. These combined findings suggest that agroecological farms can amplify social and ecological functions of agroecosystems to achieve win-win outcomes following a transition period.

Correspondingly, the third and final major finding from this dissertation is that the ecological and socioeconomic co-benefits of agroecological transitions do not manifest immediately. In fact, in Chapter 2 we found that farms in transition with five or fewer years of experience had lackluster socioeconomic outcomes, including lower farm incomes and increased work difficulty, compared to both established agroecological and conventional farms in our study. Similarly, while farms in transition brought 6-fold more crop and livestock species to market than conventional farms in the region, they tended to rely on an input substitution management approach rather than the more transformative system re-design approaches (e.g., agroforestry and crop-livestock integration) used on agroecological farms. Differences between transitioning and established agroecological farms' management also related to ecological performance. Farms in transition had rates of potentially mineralizable N (an indicator of soil nutrient availability and the quality of soil organic matter) two times lower than agroecological farms, as well as lower mean soil organic C and total N. This finding suggests that further policy support for capacity building, marketing, and financing for farms transitioning to agroecological management may be needed during this period. Incentives could enable more farms to gain the knowledge and skills necessary to achieve the social-ecological synergies apparent on agroecological farms in this study.

Cumulatively, the results of this dissertation can inform future process-based analyses that seek to compare outcomes of different production systems or management practices. For instance, our case study in southern Brazil attests that the length of time a farm has used a particular practice or set of practices can have major implications for its ecological and socioeconomic performance. Future studies would likely gain a more complete, mechanistic understanding of different systems by grouping farms based not only on their management regime (e.g., Teixeira *et al.*, 2020) but also on the length of time or level of experience with the management practices of interest. As demonstrated in this dissertation, analyzing outcomes along a transition gradient is one promising approach. This analytical method could be a particularly useful way to partition data when conducting meta-analyses on organic, regenerative, or sustainable agriculture, given the highly variable management systems and social-ecological functions provisioned by farms within each of those broad categories (Seufert and Ramankutty, 2017).

5.2 Social-ecological context and generalizability of results

Agroecological transitions do not take place in a vacuum. They are structured by both social and environmental conditions and ultimately arise from the agency of farmers who choose to undertake them. It follows, then, that the results of this dissertation depend to some extent on the specific social-ecological context in which the three studies were conducted, and further research is required to ascertain whether the patterns identified in southern Brazil hold true in other agricultural regions and along distinct socio-environmental gradients.

Social context and implications

In several ways, the social context of my dissertation research makes the findings of Chapters 2 and 3 equivalent to a "best case scenario" for the outcomes of agroecological transitions, at least under current policy frameworks. One contextual factor that strongly influenced the results of Chapters 2 and 3 was the role of the participatory certification network Rede Ecovida in the study region. Rede Ecovida's influence meant that farms included in this research were not only certified organic; they were certified "agroecological." Farm households were embedded in a network and movement that facilitates ecological and marketing knowledge for diversified production, features semi-annual workshops on crop and livestock diversification and other agroecological practices (e.g., agroforestry, composting), and aggregates agroecological produce for sale across more than four Brazilian states. Farmers are also encouraged to continue diversifying and improving their ecological management practices over time, beyond the bounds of the official two-year transition period to become certified agroecological (Rede Ecovida de Agroecologia, 2004). Rede Ecovida provides many of the resources that previous research has identified as conducive to successful agroecological transitions, including a supportive peer network, educational opportunities, and access to markets to sell diversified produce (Guerra et al., 2017; Home et al., 2017; Niederle et al., 2020). The network's influence thereby provided an opportunity to evaluate transitions where they are likely to embody agroecological principles.

Furthermore, what Rede Ecovida does not provide, other policy instruments in Brazil do. There are multiple national programs active in southern Brazil that provide guaranteed, government-mediated markets for regional produce, with price premiums and preferential contracts for agroecological producers (Guerra et al., 2017; Valencia et al., 2019). Rede Ecovida's system of participatory organic certification is also recognized at the federal level, elevating the marketing opportunities available to agroecological farmers who wish to market their produce as "organic" (Sacchi et al., 2015). Growing market demand for agroecological produce (EPAGRI, 2019) has further enabled agroecological transitions for farmers in the region, leading them to perceive agroecological farming as a promising investment with high potential returns and relatively low risk. Farms in the region tend to be medium to large in size and are owned by family farmers of European descent who colonized the area in the 19th century (Wolford, 2010); as such, farms tend to be labor- rather than land-limited. Another environmental policy, the Brazilian Forest

Code, requires that 20% of agricultural land remain in natural vegetation, meaning that all farmers maintain forest cover as a component of their total agricultural area, potentially increasing ecosystem functioning in the region more broadly. Each of these social factors has potential to encourage farmers in southern Brazil to transition to agroecological management.

Taken together, the amenable social networks, resources, markets, and policy environment for agroecological transitions in southern Brazil suggest that the agroecological farms in this dissertation may have outcomes above and beyond what would be considered typical in other global regions. I chose to conduct my dissertation research in southern Brazil for this very reason; the beneficial policy environment provides a valuable window into what could be possible for agroecological transitions if similar sustainable farming policies were implemented elsewhere. The win-win social and ecological outcomes from this case study endorse this confluence of food systems policies and farmer-led institutions as a model for other regions aiming to facilitate agroecological transitions. To assess the generalizability of these outcomes, additional empirical and modeling studies could explore how transitions affect social, ecological, and nutritional functions of agroecosystems under distinct socioeconomic and political conditions.

Ecological context and implications

Unlike the positive social features described above, the environmental conditions in eastern Santa Catarina, Brazil, prove a challenging backdrop for agriculture. The region's acidic, low-fertility soils and hilly topography result in lower yields and more labor-intensive management regimes than farmers in other regions may have to endure (EMBRAPA, 2004; Wildner et al., 2004). Moreover, the historical and current prevalence of intensive tobacco cultivation has further degraded many agricultural soils in the region. Each of these environmental factors played a role in our results in Chapters 2 and 3, and we can draw lessons from each of them to predict how crop diversification practices may have differing effects on ecological and nutritional functions of agroecosystems in other contexts.

Most importantly, effects of cover cropping and intercropping with legumes may have been limited by soil acidity on the majority of farms in Chapter 3 (to varying degrees) and on the experimental farm in Chapter 4. Total N fixation by the vetch cover crop on farms across the transition gradient was quite low on average (means: 20-50 kg/ha) (Ladha and Peoples, 1995), particularly in the first year of the experiment. This suggests that the results presented in this dissertation likely underestimate the potential impacts of legume-based crop diversification on soil N cycling in more calcareous or higher fertility soils. On the other hand, Santa Catarina, Brazil, has a subtropical climate with ample rainfall during and after the cover cropping period. In more arid climates that rely on rainfed agriculture, cover crops may not have consistently positive effects on crop yields, as they can limit water availability for subsequent crops (Pinto et al., 2017). Future research is needed to evaluate the water and nutrient cycling effects of combined diversification practices, including cover cropping, intercropping, and perennial agroforestry systems, along distinct climate and soil gradients.

Seeing as farms in the first 5 years of transition may need the most support to maintain their socioeconomic viability, future studies on crop diversification practices could aim to maximize agroecosystem functions on farms within this period. While I chose to test two practices that increase functional diversity within an annual crop rotation, recent research underscores that perennialization of crop rotations could have stronger effects on the accumulation of soil organic C and other ecological functions of agroecosystems (King and Blesh, 2018; Ryan et al., 2018). Future studies that seek to enhance agroecosystem multifunctionality could therefore consider testing functionally diverse crop mixtures that incorporate perennials. Examples include the use of cover crops in early successional agroforestry systems (Santos et al., 2021) and integration of perennial legumes and grains into rotations (e.g., intermediate wheatgrass and alfalfa intercrop) (Ryan et al., 2018).

5.3 Future research, from the agroecosystem to the food system

Integrating qualitative and quantitative social, ecological, and nutritional data, this dissertation has focused on processes and outcomes of agroecological transitions at the farm, or agroecosystem, scale. This is the scale at which farm management decisions are made and thus provides a useful frame for understanding transition processes from farmer perspectives. However, agroecosystems are one component of the larger food system, and both the mechanisms and results of agroecological transitions are influenced by macro-level processes that I did not consider in this dissertation. To conduct generalizable tests of the hypotheses generated in my two-year long experiment at the agroecosystem level, future research is needed at larger spatial and temporal scales.

As a first step to expand the spatial scale of analysis, comparative studies between regions could determine the extent to which the win-win social, ecological, and nutritional effects of crop diversification identified here are apparent under distinct governance and environmental conditions. The agroecological transition index developed in Chapter 2 and implemented in Chapter 3 of the dissertation could be applied to larger groups of farms to assess patterns across gradients of interest. Specifically, ecological functions including biological N fixation, soil N cycling, and crop productivity could be measured across agroecological transition gradients in differing environmental contexts. Comparable datasets from distinct regions could be combined to generate the larger sample sizes needed to account for numerous co-variates, which could be accomplished using novel online farm management platforms such as LiteFarm (https://www.litefarm.org/). Socioeconomic outcomes of agroecological transitions, including working conditions and farm income, could be examined in regions with contrasting levels of policy support (e.g., with or without the influence of a farmer network, incentives programs, or mediated markets), resources (e.g., access to credit, farm size, land tenure, labor availability), or market conditions (e.g., market access, consumer demand for agroecological products). Such analyses could help to disentangle the multiple structural and social factors that affect transition processes and outcomes.

The transition gradient proved to be a useful cross-sectional approach to understand different outcomes across farms with comparable management histories, soil types, and climate conditions. When testing diversification practices on farms, measuring any of the outcomes from Chapters 2, 3, and 4 over longer time periods would likely yield rich panel data with potential to identify stronger causal relationships than could be measured with a gradient approach in just two years. When such detailed data is not accessible, studies over larger spatial scales could utilize a “before-after-control-impact” study design that assesses differences in outcomes over time between farms that have undergone agroecological transitions and those that have maintained their prior management regime (Underwood, 1994; Kremen and M’Gonigle, 2015).

Causal inference methods such as propensity score matching with covariate balancing across regions could also be used for a more robust approach (Dyngeland et al., 2020).

In this dissertation, I conceptualized "nutritional functions" in terms of nutrient production at the agroecosystem level (Figure 1.1, Table 1.1). It is important to acknowledge, however, that broader analyses at the food system level would be required to evaluate the relationship between agroecosystem functions and food security or nutritional adequacy of diets for individuals or populations. Future research could explicitly examine the link between nutritional functions of agroecosystems, such as crop nutrient content or nutrient yields across all crop and livestock species, and nutrient adequacy of diets in specific populations (Hatloy et al., 1998; Arimond et al., 2010). Nutrient adequacy of diets provides a more complete measure of food security, as it integrates availability, access, utilization, and stability of food consumption if measured at multiple time points (FAO, 2008; Arimond et al., 2010). Such analyses could determine whether crop and livestock diversity or functional diversity of agroecosystems are important predictors of dietary adequacy at multiple spatial scales, from the agroecosystem to the food system at the community, regional, or national level. Multiple nutritional functions, including nutritional functional diversity of entire cropping systems (Luckett et al., 2015; Wood, 2018), could also be measured across an agroecological transition gradient to understand spatiotemporal patterns of nutrient production driven by farm management decisions.

As a final note on nutrients, I encourage future research across disciplines to consider the fact that crop nutrient content is not accurately represented as a single, static value for each species or variety. As Chapter 4 of this dissertation demonstrates, crop nutrient content varies not only along environmental gradients (Wood et al., 2018; Gashu et al., 2021), but also based on farm management practices (Zuo and Zhang, 2009). Relative to low-input monocultures, I found that diversified farming systems can yield crops with higher nutrient content. Such patterns are important to consider when modeling impacts of agricultural management on food availability (e.g., based on crop yields: Morais *et al.*, 2021) or diet quality (Willett et al., 2019), as is increasingly common in the sustainable diets literature. Rather than relying on mean values from food composition tables, modeling studies could estimate nutrient output for different agroecosystems using distributions of crop nutrient content and yields, both of which shift based on soil and management characteristics.

In closing, agroecological transitions are complex, social-ecological processes that aim to increase the sustainability of agricultural systems. The broader social-ecological context provides a structure that can enable or constrain a farm's ability to transition, and can mediate the agroecosystem functions it provides. At the agroecosystem level, transition outcomes depend on the interactions between background soil characteristics, farm management histories, and current management decisions, including use of diversification practices. This dissertation elevates the notion that diversified, agroecological farms can achieve socioeconomic viability while bolstering key ecosystem functions including soil nutrient cycling. Furthermore, the functional diversity they employ can also lead to more nutrient-rich crops. The evidence from southern Brazil is clear: in five years or less, agroecological transitions within a supportive policy environment can foster social, ecological, and nutritional synergies in agriculture. Given the pace of environmental change, this dissertation prompts the question: what are we waiting for?

Appendices

Appendix 1: Supplemental Material for Chapter 2

A1.1 Supplemental methods

Quantification of ecological management indicators

For indicator 1, *crop and livestock diversity,* we calculated three measures of crop diversity on each farm—crop species richness, Shannon diversity index, and Simpson's diversity index—for both total farm cultivated diversity as well as for marketed crops only (which made up a higher proportion of total cropped area). Both Shannon and Simpson's diversity indices were weighted by area and calculated using the diversity() function in the *vegan* package in R (Oksanen et al., 2019; R Core Team, 2019). Because its range compares to other management indicators (0-1), Simpson's diversity index was used for indicator 1. Indicator 2, *continuous soil cover*, was calculated as the percent of the cultivated or managed farm area with any of the following land uses: perennial pasture, agroforest, eucalyptus plantation, or annual crops with cover crops in the fallow period. Indicators 3 and 4 represent two categories of managing ecological processes on farms: for soil fertility and for weed and insect (pest) control. They were calculated through the same process, as follows. Farmers were asked how they managed fertility and pests for each crop type in a given field, and each management practice was categorized as either synthetic (e.g., glyphosate herbicide) or ecological (e.g., hand weeding, use of cover crops to suppress weeds). An index of ecological management was then calculated for each crop species per field, based on a scale of 0-1, where 1 was equal to 100% ecological management and 0 was equal to 100% synthetic management for a given crop. All crops for which no chemical methods of pest control or nutrient management were employed were given a score of 1. Next, we calculated the proportion of ecological management for each field and farm, weighted by cultivated area. We used this method to quantify *ecological nutrient management* (indicator 3), and *ecological pest management* (indicator 4). The latter was calculated as the average of ecological weed management and ecological insect and disease management.

Quantification of socioeconomic indicators

Financial independence

To reduce reporting bias in our calculations of average annual agricultural income, we asked farmers about their household finances at two timepoints and focused on a short recall period (e.g., monthly rather than annual) (Beegle et al., 2011). We also asked farmers to report agricultural income both on a monthly basis and based on crop sales from their principal 3 crops, as asking about specific events such as crop sales can improve recall data quality (Beegle et al., 2011). Due to the diversity of crops grown on farms in the sample, the incomes based on sales of only three crops were not representative, and the monthly average was selected as a more accurate measure of gross agricultural income. Monthly values were used to estimate annual incomes. Off-farm income, including retirement pensions (on 4 farms), was reported for all working household members. Expenses were calculated separately by type of agricultural input and household expense, including payments on agricultural investments in machinery and infrastructure, and were then aggregated into annual values for agricultural and non-agricultural household expenditures. All standard measures of income are represented in our calculations of net household income except in-kind earnings from home consumption of agricultural products and depreciation of equipment (Grosh and Glewwe, 2000). All monetary values were first calculated at the monthly level in Brazilian reais, extrapolated to the annual level, and converted to USD assuming a 4 BRL to 1 USD exchange rate (the average 2019 rate was 3.946). We interpreted these quantitative values within the context of farmers' qualitative explanations of changes in income on their farms in our analysis.

Market access

We calculated two complementary measures of market access: number of marketing channels and the percentage of gross annual income derived from the highest earning crop (as a proxy for single-market dependence). Marketing channels were the different avenues through which farms sold their fresh produce and value-added products. We asked farmers what proportion of their total agricultural income came from the following sources: government programs (PAA or PNAE), farmers markets, intermediaries/wholesalers, cooperatives, direct sale to other farmers, direct sale to relatives or friends, sale to supermarkets and stores, and community supported agriculture (through vegetable baskets, cooperative buying schemes, or local institutional sales). We calculated the percentage of annual gross household income from the highest earning crop using farmer-reported yield and price data to calculate annual earnings from that crop, then dividing by total gross annual income for each household.

Working conditions

We used three indicators of *working conditions* on farms: working hours, occupational health, and work quality. We calculated *working hours* for agricultural and off-farm work. In interviews we asked farmers the number of months per year, weeks per month, days per week, and hours per day each household member worked. We then asked what proportion of each worker's time was allocated to agricultural activities, farm-related activities (e.g., crop processing for value-added products or product transport to farmers markets), off-farm work and other professions, and domestic work. We took extra care to include domestic tasks such as processing farm products and meal preparation for the household, as well as direct agricultural or marketing work, in the counts to accurately represent women's work. Total hours of agricultural work (the sum of agricultural activities, farm-related activities, and domestic labor) and off-farm work were calculated separately for each individual on an annual basis and then aggregated across household members to determine total hours of work per farm household. We then calculated annual per capita working hours per farm by dividing the total hours of work by the number of working household members. Qualitative data from interviews were used to assess *occupational health* and *work quality*, following Dumont and Baret (2017), using thematic coding exercises in NVivo software (QSR International).

A1.2 Supplemental socioeconomic indicators

Table A1.1. Additional socioeconomic indicators on farms at different stages of transition in Santa Catarina, Brazil. Bolded rows show the indicators of income and working conditions used in our main analyses, also shown in Table 2.1 and Table 2.3.

Indicator type	**Indicator**	**Conventional**		**Transition**		**Agroecological**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Financial independence	Gross annual agricultural income (USD)	$66,248	$62,673	$10,079	$5,826	$12,975	$6,525
Financial independence	Gross per capita annual agricultural income (USD)	$11,208	$10,286	$2,517	$1,715	$4,390	$3,365
Financial independence	Annual agricultural expenditures (USD)	$55,129	$64,292	$5,157	$3,037	$6,483	$3,001
Financial independence	Net annual agricultural income (USD)	$11,119	$7,759	$4,922	$6,277	$6,492	$4,899

Financial independence	**Net per capita agricultural income (USD)**	$1,964	$1,351	$1,241	$1,403	$2,102	$1,874
Financial independence	Annual off-farm income (USD)	$2,250	$4,500	$7,044	$6,774	$8,840	$5,145
Financial independence	Gross income from off-farm sources (%)	$1	$3	$38	$36	$41	$24
Financial independence	Gross annual (total) household income (USD)	$68,498	$67,090	$17,123	$5,061	$21,815	$7,021
Financial independence	Net annual (total) household income (USD)	$13,369	$8,862	$11,966	$4,046	$15,332	$4,324
Financial independence	**Net per capita household income (USD)**	$2,339	$1,491	$2,762	$902	$5,284	$3,459
Financial independence	Number of sales channels	2	1	3	2	3	1
Financial independence	Gross annual agricultural income from most lucrative crop (%)	59	40	33	19	33	19
Working conditions	Annual agricultural working hours	15769	5165	8803	5136	7903	2907
Working conditions	**Annual per capita agricultural working hours**	2830	684	2150	252	2234	590
Working conditions	Annual off-farm paid working hours	0	0	864	1118	902	1462
Working conditions	Annual per capita off-farm paid labor hours	0	0	210	254	339	503
Working conditions	Total annual working hours (on- and off-farm)	15769	5165	9667	5624	8805	3119
Working conditions	**Total annual per capita working hours (on- and off-farm)**	2830	684	2360	296	2573	920

Working conditions	Total weekly per capita working hours	59	14	49	6	54	19
Working conditions	Number of workers	6	1	4	3	4	2
Working conditions	Number of household members	6	1	5	2	3	1

Appendix 2: Supplemental Material for Chapter 3

A2.1 Agroecological management index

We calculated the agroecological management index following a methodology used in a social science companion paper from this study (Chapter 2). For the present analysis, we used land use history data from farmer interviews in 2018 to calculate an index for the specific field on each farm where our ecological study was conducted. The index is the sum of four indicator scores, each of which assesses fields' management conditions directly prior to beginning the experiment, as follows: (1) crop and livestock diversity, (2) continuous soil cover, (3) nutrient management, and (4) pest management. All scores range from 0 (least ecological) to 1 (most ecological) and the sum of the four scores is equal to the agroecological management index for one experimental field. For crop and livestock diversity, fields' scores are equivalent to Simpson's diversity index based on the proportional abundance of cultivated species in the field. We used the "vegan" package in R to calculate Simpson's index using cropped area to weight species abundance (Oksanen et al., 2019). For permanent soil cover, indicator scores were calculated based on the proportion of the field under continuous living soil cover in the year prior to the study (i.e., perennial crops, annual crops followed by cover crops). Scores for nutrient and pest management indicators were both calculated as the proportion of the field managed using ecological methods (e.g., manure and compost application for nutrient management, or biocontrol practices for pest management) relative to synthetic chemical or conventional methods (e.g., mineral fertilizer application for nutrient management, or insecticide application for pest management) in the year prior to the study. For a more detailed description of the calculation procedure and what practices were included in each indicator, see Appendix 1.

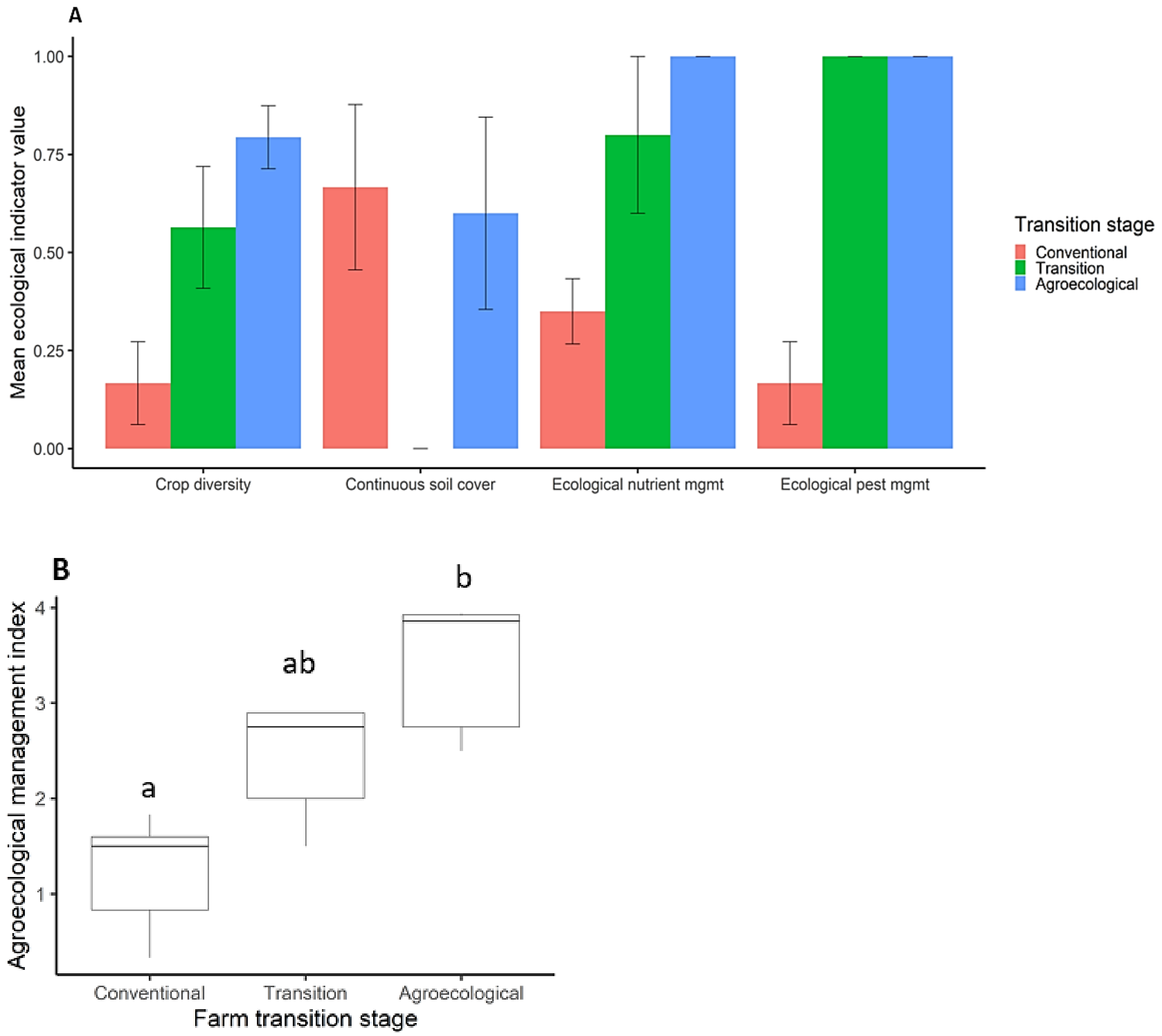


Figure A2.1. A: Bar graph showing mean ecological indicator scores (± standard error) in experimental fields by agroecological transition stage (conventional, transition, and agroecological). Indicators are shown in order, as follows: (1) crop and livestock diversity, (2) continuous soil cover, (3) ecological nutrient management, and (4) ecological pest management. With the exception of indicator 2 (continuous soil cover), indicators tended to increase from conventional to agroecological farms' fields. **B:** Boxplots showing that the agroecological management index (calculated as the sum of all indicators per field) significantly increased from the conventional to the agroecological transition stage (significant difference from "a" to "b": p=0.005 by Tukey's post-hoc test) but transitioning farms did not differ from other stages.

A2.2 Soil and climate variables

Methods for climate data aggregation

Climate data were downloaded from the Santa Catarina state agricultural agency website for each farm's municipality over the full study duration (EPAGRI 2020). For each municipality, we calculated mean monthly precipitation (cumulative mm/number of months in cropping season) and mean monthly temperature (average daily temperature across months in cropping season) for both the winter cover crop (May-August) and the spring intercrop (September-December) seasons in 2018 and 2019 (Table 3.1). Where data did not exist for a given municipality, we used the most proximate municipality with similar altitude and climate conditions as a proxy.

Detailed methods for soil analyses

Baseline soil characteristics

SOM was obtained using a visible spectrophotometer after sulfuric dichromate wet oxidation at 70 °C. Soil micronutrients Mg, Ca, Al, and Mn were extracted in 1 M KCl and diluted with 1% HCl prior to analysis on an atomic absorption spectrometer (Analytik Jena contrAA-700); macronutrients P and K were extracted with a Mehlich-1 solution and determined by flame emission spectrophotometry and photocolorimetry (Digimed DM-63); and micronutrients Fe, Zn, and Cu were extracted with a Mehlich-1 prior to reading on an atomic absorption spectrometer.

Potentially mineralizable N (PMN) determination

Composite soil samples of 10 cores each (20-cm deep) were collected on cover crop and fallow sides of each farm field (with duplication of sampling on 5% of fields for quality control), stored under refrigeration for up to 48 h, and homogenized prior to analysis. Immediately following sampling, gravimetric soil moisture was determined by drying samples for 48 h at 105 ºC. Triplicate soil subsamples were sieved to 2 mm and extracted with 2 mol/L KCl to determine extractable inorganic N ($NO_3^- + NH_4^+$) prior to the incubation. Additional duplicate subsamples of 20 g of fresh soil were separated, sieved to 4 mm, and brought to 50% water-filled pore space (WFPS) with DI water using the following calculation, modified from Haney and Haney (2010) for field-moist soil:

WFPS = (Soil water content × Bulk density)/ [1 – (Bulk density / Particle density)], where soil water content is equivalent to gravimetric soil moisture in 4 mm sieved soil, bulk density is calculated as the weight per 5 mL volume of fresh soil sieved to 4 mm after accounting for soil moisture, and particle density is a constant of 2.65 Mgm^{-3}. Subsamples were then incubated aerobically in sealed jars (without purging the headspace) for 14 days in a dark environment at 21.5 ºC. Following incubation, triplicate samples were sieved to 2 mm and extracted with 2 mol/L KCl, as above. The quantity of NH_4^+ and NO_3^- in all samples was determined colorimetrically using a discrete analyzer (AQ2, Seal Analytical, Mequon, Wisconsin, USA).

Baseline physical and chemical soil characteristics

Table A2.1. Mean values for physical and chemical soil characteristics for fields in the on-farm experiment. All values are from baseline soil samples collected between February 28-March 30, 2018 for farms that participated in the first year of cover cropping, or from September 5-10, 2018, on farms that did not have cover crops in the first year. One exception is soil moisture, which was measured in samples collected following the second year of cover cropping, from September 24-October 2, 2019. Listed pH is the buffered value.

Farm ID	Texture class	Sand (%)	Clay (%)	pH	Bulk dens-ity (g/cm3)	P (kg/ ha)	K (kg/ ha)	Ca (mg/ L)	Mg (mg/ L)	Zn (mg/ L)	Fe (mg/ L)	Soil moisture (%)
1	clay	15	51	6.2	1.20	13	273	1892	1404	3.2	134	20
2	clay loam	35	31	5.3	1.04	11	126	1073	956	2.3	419	31
3	sandy clay loam	54	27	6.1	1.24	76	390	1112	644	2.5	359	22
4	sandy clay loam	50	27	6.0	1.12	39	129	605	410	1.9	199	25
5	sandy clay loam	53	26	6.1	1.15	53	318	1560	878	2.5	311	25
6	sandy clay loam	49	35	5.8	1.17	46	621	1346	1014	2.5	242	18
7	sandy clay loam	59	36	4.9	1.12	20	166	624	332	1.8	185	16
8	sandy clay loam	53	30	6.2	1.36	11	283	1599	722	2.9	229	18
9	sandy clay loam	53	24	6.4	1.23	139	881	1892	956	9.0	193	23
10	clay loam	42	36	5.0	0.86	6	207	234	156	1.9	296	26
11	sandy clay loam	53	25	6.6	1.16	343	980	3276	1404	16.2	73	14
12	clay loam	35	34	5.8	0.99	24	203	1677	1346	2.7	165	23
13	clay	14	50	5.7	0.88	20	509	2496	1541	4.3	180	25
14	clay loam	44	27	6.7	1.38	109	724	2223	995	7.3	53	17

Baseline farm management history and biological soil characteristics

Table A2.2. Summarized management histories and soil biological properties of fields in the on-farm experiment. All values represent baseline conditions (2018). PMC = potentially mineralizable carbon. The agroecological management index represents a scale from 0 (least ecological) to 4 (most ecological) across indicators of crop and livestock diversity, continuous soil cover, and pest and nutrient management (Appendix A2.1, Chapter 2).

Farm ID	**Transition stage**	**Years certified organic**	**Agroecological management index**	**Total organic C (Mg/ha)**	**Total N (Mg/ha)**	**Soil C:N**	**PMC (μg CO_2-C/g/d)**
1	Conventional	0	1.75	34.9	4.7	7.5	43.0
2	Conventional	0	2.19	83.2	7.1	12.0	67.9
3	Conventional	0	1.04	30.3	3.0	10.5	68.8
4	Conventional	0	1.29	31.9	3.0	10.0	57.1
5	Conventional	0	2.71	31.2	2.7	12.0	34.0
6	Transition	3	3.57	47.7	3.8	12.5	63.9
7	Transition	0	2.56	34.6	2.9	12.0	42.0
8	Transition	5	3.74	45.0	3.4	13.0	46.1
9	Transition	3	2.71	44.7	3.9	11.5	88.6
10	Agroecological	7	3.65	44.2	3.6	12.0	75.0
11	Agroecological	8	3.85	53.8	4.6	12.0	102.4
12	Agroecological	9	3.86	57.1	4.5	13.0	107.0
13	Agroecological	12	3.88	49.0	4.7	11.0	93.5
14	Agroecological	20	3.53	40.5	4.1	10.0	65.4

A2.3 Quantifying vetch N fixation

In 2018, subsamples of aboveground biomass from common vetch and non-legume weeds were used for biological N_2 fixation (BNF) analysis, as treatment and reference plants, respectively. In 2019, we planted two additional 1 m^2 black oat monoculture plots several meters from the cover crop treatment on each farm, and black oat from these plots served as the reference species for the natural abundance method (Shearer and Kohl, 1986). In 2019, we collected vetch leaf material from three representative plants (~1 gram per plant) in the oat-vetch cover crop mixture and from the two oat monoculture plots. BNF samples were collected at peak vetch flowering, attempting to capture maximum N_2 fixation rates. Leaves from legume and reference samples were finely ground using a cyclone mill and analyzed for ^{15}N and total N content using a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis, California, USA). BNF was calculated using the mixing model:

$$\% \text{ plant N from fixation} = 100 \times ((\delta^{15}N_{ref} - \delta^{15}N_{legume}) / (\delta^{15}N_{ref} - B)),$$

where $\delta^{15}N_{ref}$ represents the $\delta^{15}N$ abundance in the non-legume reference plant (black oat or weeds), $\delta^{15}N_{legume}$ is the $\delta^{15}N$ abundance in the N_2-fixing species (common vetch), and B is the $\delta^{15}N$ signature of the legume grown in a N-free medium with only atmospheric N_2 as a N source (see below).

B value experiment for estimating legume N fixation using the natural abundance method

Vetch plants used to calculate the B value were grown in autoclaved Turface© following seed sterilization and inoculation with *Rhizobium leguminosarum* (USDA 2347; source: USDA ARS) at a rate of approximately 1.5×10^6 cells per seed. The experiment took place from April-July 2019 in a controlled, sterile environment under grow-lights at the University of Michigan and consisted of six pot replicates with two vetch plants per pot. Pots were watered daily with reverse osmosis N-free water and fertilized with N-free fertilizer twice per week. When vetch plants reached 50% flowering, leaves were harvested following the same protocol as the field vetch BNF sampling in 2019 and also analyzed for ^{15}N and total N content at the UC Davis Stable Isotope Facility.

A2.4 Principal Components Analysis (PCA)

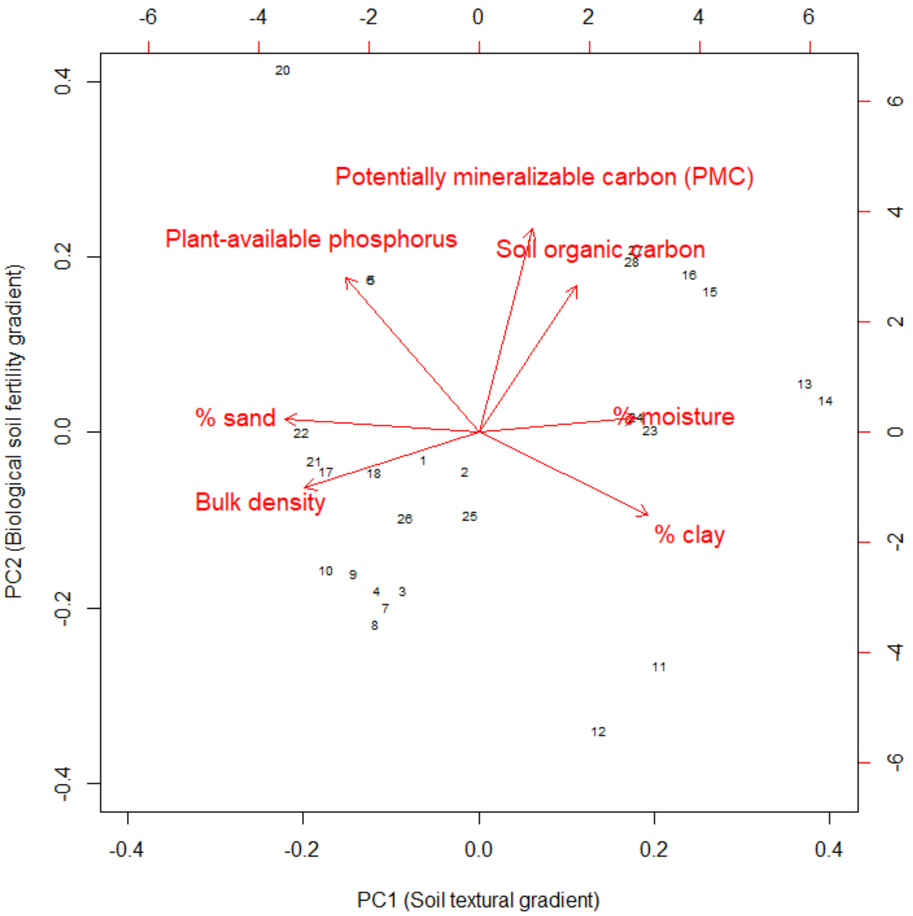


Figure A2.2. Biplot from a Principal Components Analysis (PCA) of variables related to agricultural soil fertility. PC1 in the analysis includes soil variables that are not sensitive to management and represents a soil textural gradient, ranging from sandy, dense soils with negative loadings to clayey, moist soils with positive loadings. PC2 is primarily composed of biological and chemical indicators of soil fertility, with potentially mineralizable carbon (PMC) and soil organic carbon (SOC) most strongly positively loaded on this axis. We therefore considered PC2 to represent a biological soil fertility gradient. Together, PC1 and PC2 account for 65% of the variance in soil variables across farms in the study.

To test our hypotheses related to soil fertility across farms, we extracted composite soil variables with eigenvalues greater than 1 from a PCA (Figure A2.2). The first two PC axes were used as predictors in diversification outcome models (i.e., structural equation models (SEMs)). pH was analyzed separately from the other soil variables because it is a factor that can be equally affected by management and soil parent material and other physical attributes and therefore had high loadings on both PC1 and PC2 before it was removed from the PCA. Additional soil variables (e.g., soil Ca, total soil N, plant-available K) were included in the first version of the PCA but were subsequently excluded due to high correlations with other soil variables.

A2.5 Cover crop and intercrop outcomes

Cover crop outcome variation across experimental years

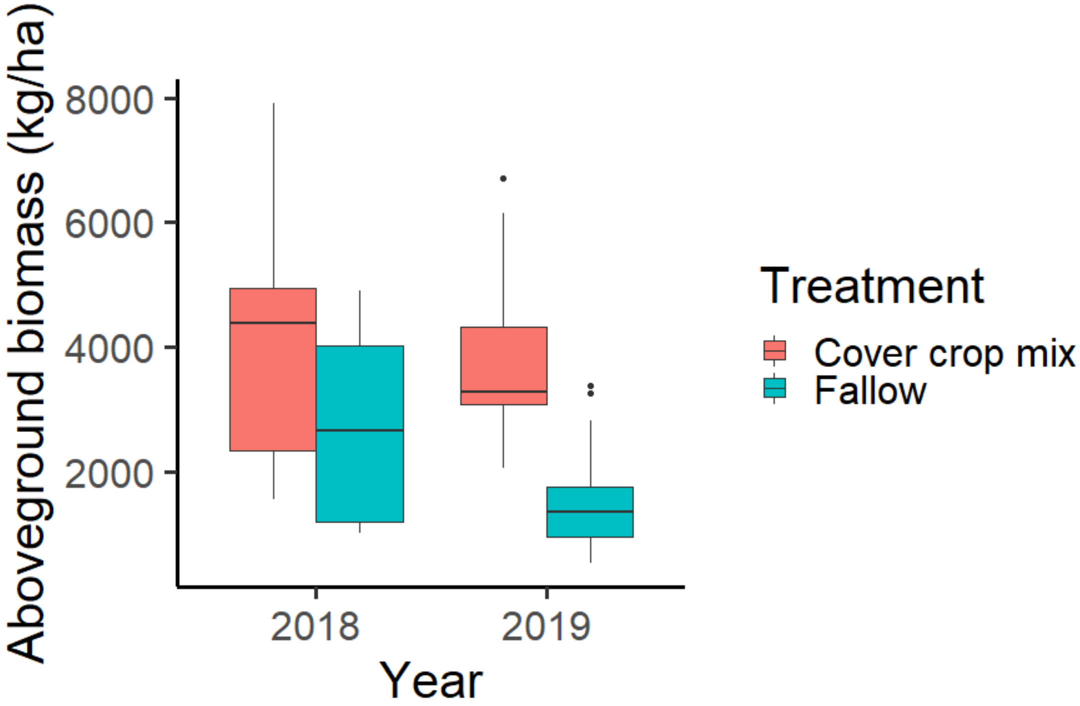


Figure A2.3. Boxplot of aboveground biomass from cover crop and weedy fallow treatments in 2018 and 2019, across all farms in the experiment (n=42). Biomass significantly differed between treatments in 2019 (p=0.001) but not 2018 (p=0.19) based on mixed effects ANOVA with year and cover crop treatment as fixed effects and farm as a random effect.

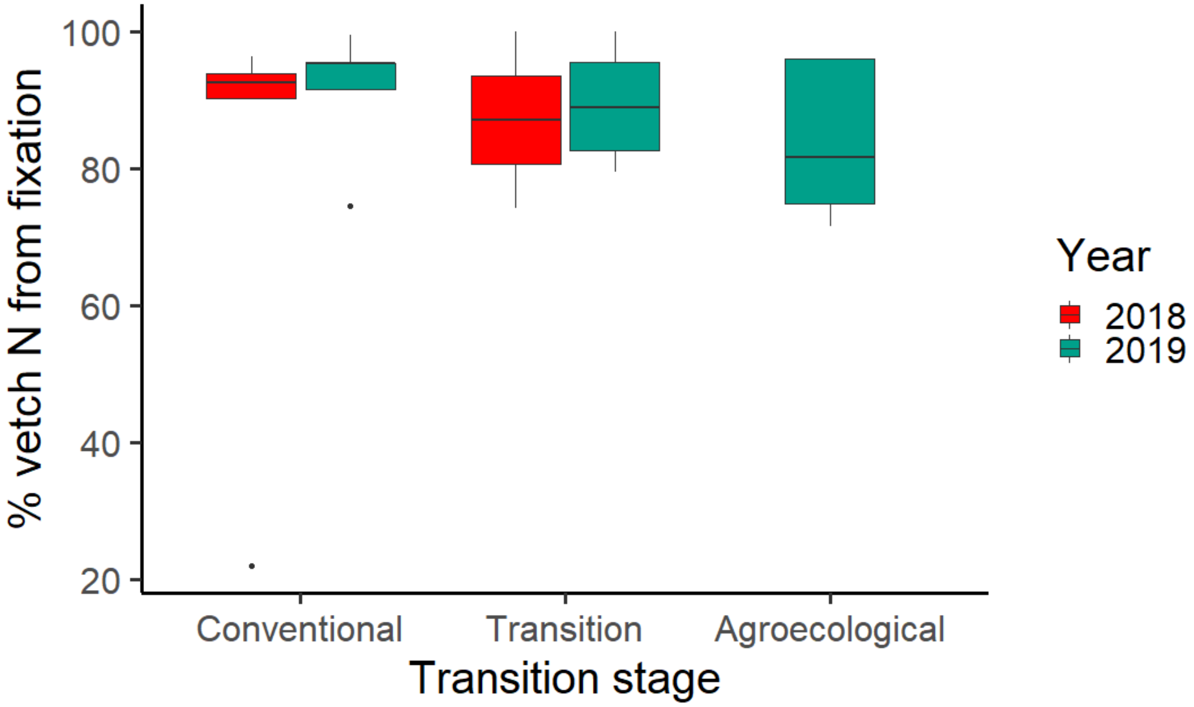


Figure A2.4. Boxplot of percent vetch N from fixation in aboveground biomass, shown by agroecological transition stage and by year of the experiment (n=21). There were no significant differences in % N from fixation by year or transition stage, based on additive mixed effects ANOVA with year and transition stage as fixed effects and farm as a random effect.

Structural Equation Model regression results

Structural Equation Model regression table

Table A2.3. Results for component models of linear mixed-effects piecewise Structural Equation Models (SEMs), including SD-standardized and unstandardized coefficients, marginal R^2, and significance levels by predictor and model. There are two types of SEMs represented: a "full" set of conceptual models using 2019 data and a "partial" SEM that uses 2018 and 2019 data but excludes soil N cycling variables. We also include results from a separate "cover crop only" linear mixed effects model that does not include fallow treatment data. Component models included in each SEM are numbered in chronological order, based on the hypothesis they are testing. H1: Model 1. H2: Models 2-5. H3: Models 6-7.

SEM	**Data-set**	**Sample size**	**Model #**	**Response**	**Predictor**	**Unstandard-ized estimate**	**SD-Standardized estimate**	**Std. error**	**Model P-value**	**Model DF**	**Marginal R^2**	**Conditional R^2**
Full	2019	28	1	Biological soil fertility	Agroecological management index	0.722	0.588	0.274	0.022	12	0.35	0.99
Full	2019	28	3	(Ln) N inputs from cover	Cover crop presence	0.563	0.374	0.214	0.022	12	0.68	0.84
Full	2019	28	3	(Ln) N inputs from cover	Cover crop N fixation	0.012	0.498	0.004	0.007	12	0.68	0.84
Full	2019	28	4	PMN	Biological soil fertility gradient	4.172	0.674	0.763	0.000	12	0.65	0.67
Full	2019	28	4	PMN	Soil textural gradient (sand-to-clay)	2.159	0.435	0.612	0.004	12	0.65	0.67
Full	2019	28	5	Soil inorganic N availability	Cover crop presence	5.837	0.589	1.145	0.000	11	0.64	0.69
Full	2019	28	5	Soil inorganic N availability	Biological soil fertility gradient	1.579	0.426	0.486	0.008	11	0.64	0.69
Full	2019	28	5	Soil inorganic N availability	Soil textural gradient (sand-to-clay)	0.876	0.295	0.389	0.046	11	0.64	0.69
Full	2019	28	6	LER	Soil pH	0.557	0.495	0.219	0.024	13	0.23	0.74

Full	2019	28	7	LER N yield	LER	1.047	0.935	0.078	0.000	13	0.88	0.88
Partial	2018 + 2019	42	1	Biological soil fertility	Agroecological management index	0.700	0.578	0.273	0.025	12	0.34	0.99
Partial	2018 + 2019	42	1	Biological soil fertility	Soil pH	0.276	0.108	0.102	0.012	27	0.34	0.99
Partial	2018 + 2019	42	3	(Ln) N inputs from cover	Cover crop presence	0.460	0.328	0.152	0.007	18	0.57	0.81
Partial	2018 + 2019	42	3	(Ln) N inputs from cover	Cover crop N fixation	0.012	0.507	0.003	0.001	18	0.57	0.81
Partial	2018 + 2019	42	6	LER	Soil pH	0.551	0.468	0.189	0.009	19	0.2	0.71
Partial	2018 + 2019	42	7	LER N yield	LER	0.981	0.914	0.069	0.000	19	0.84	0.84
Partial	Cover crop only, 2018 + 2019	20	2	Cover crop N fixation	Soil pH	43.596	-	9.912	0.001	11	0.65	0.78
Partial	Cover crop only, 2018 + 2019	20	2	Cover crop N fixation	Year	36.831	-	6.949	0.002	6	0.65	0.78

Hypotheses supported by Structural Equation Models, shown using specific variable names

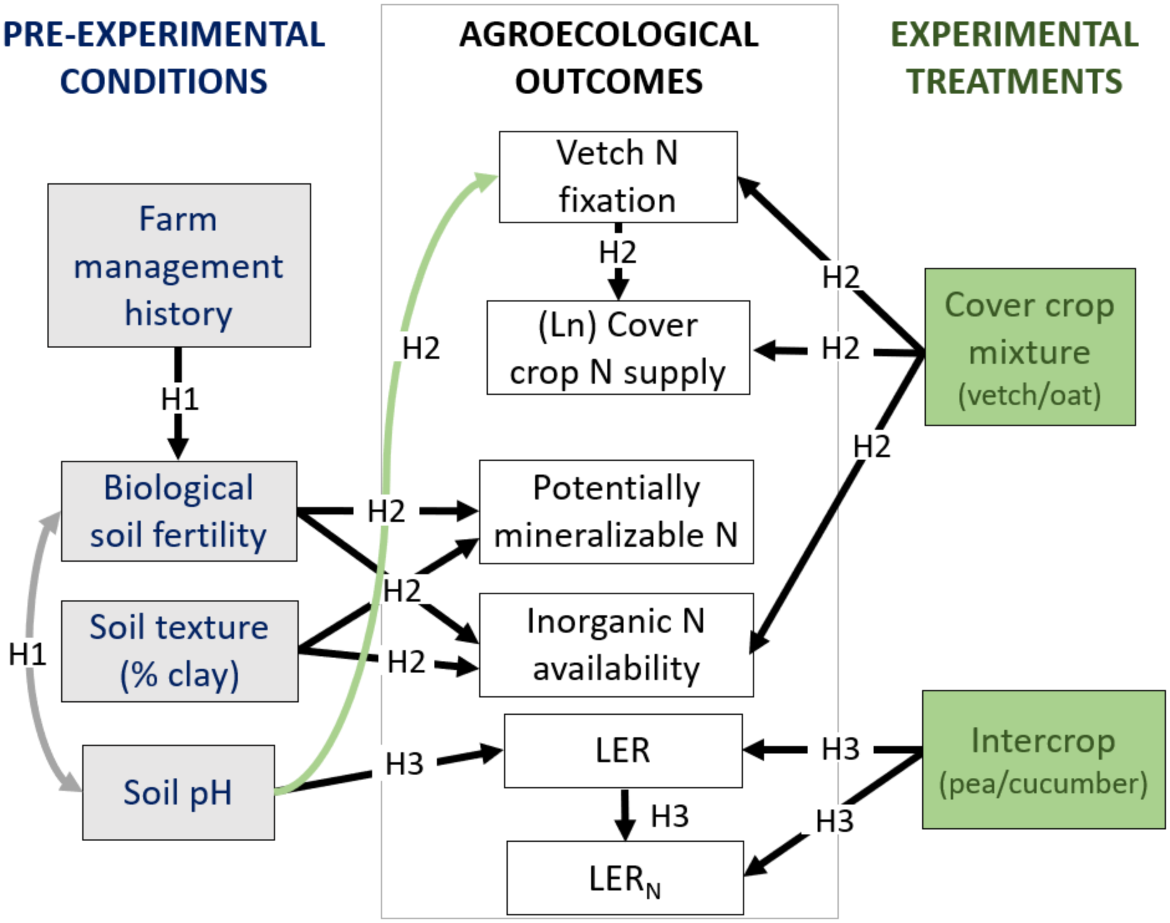


Figure A2.5. Final path diagram showing hypotheses supported by Structural Equation Models (SEMs). Gray boxes represent pre-experimental variables; green boxes represent experimental treatments; and white boxes represent measured outcomes. Solid arrows between boxes indicate positive, directed relationships supported by the data. Double-headed arrows arrows indicate undirected relationships, or correlations. Black arrows indicate relationships supported by the full SEM; gray arrows were supported by the partial SEM model; green arrows were tested using a cover crop only model (excluding data from fallow treatments). Hypotheses are superimposed on their corresponding arrows.

Fitted regression partial plots

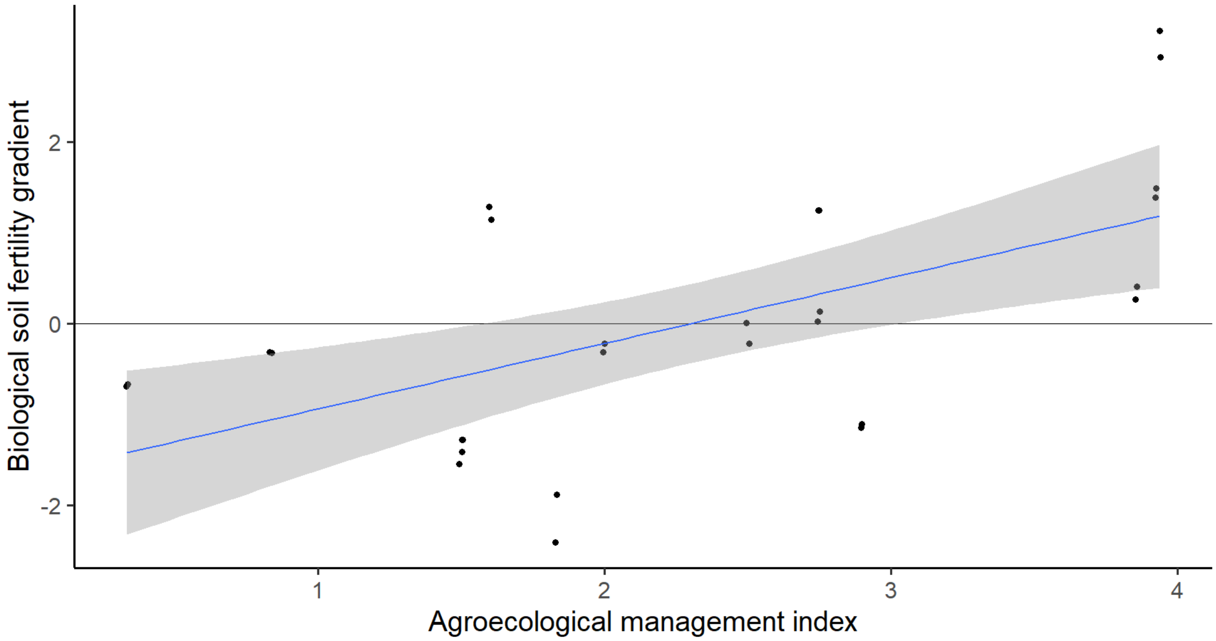


Figure A2.6. Fitted regression plot for Model 1 in the full piecewise SEM (2019 data). Relationship between the agroecological management index and biological soil fertility, including data from both sides of the experimental field. Standard error is shown in gray. Regression statistics are in Table A2.3.

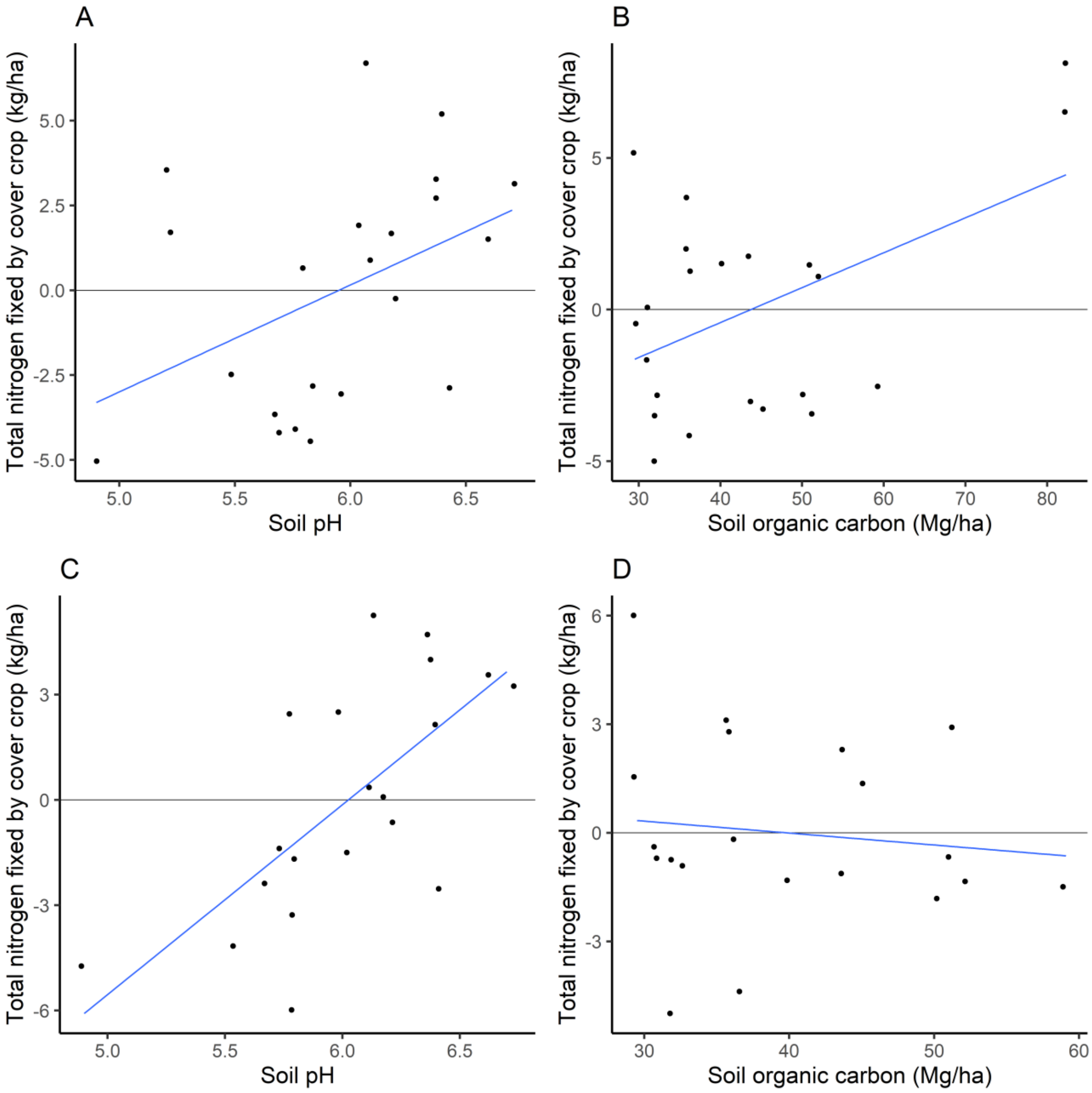


Figure A2.7. Partial regression plots for Model 2 in the "cover only" linear mixed model. No data from fallow treatments was included in the analysis. A and B: Predictors of total aboveground N fixed by the legume cover crop, modeled using the 2018 + 2019 dataset, including soil organic carbon (SOC) outliers. C and D: Predictors of total aboveground N fixed by the legume cover crop, modeled after excluding SOC outliers. After removing outliers, SOC was no longer a significant predictor of vetch N fixation; SOC was therefore excluded from the final model. Regression statistics can be found in Table A2.3.

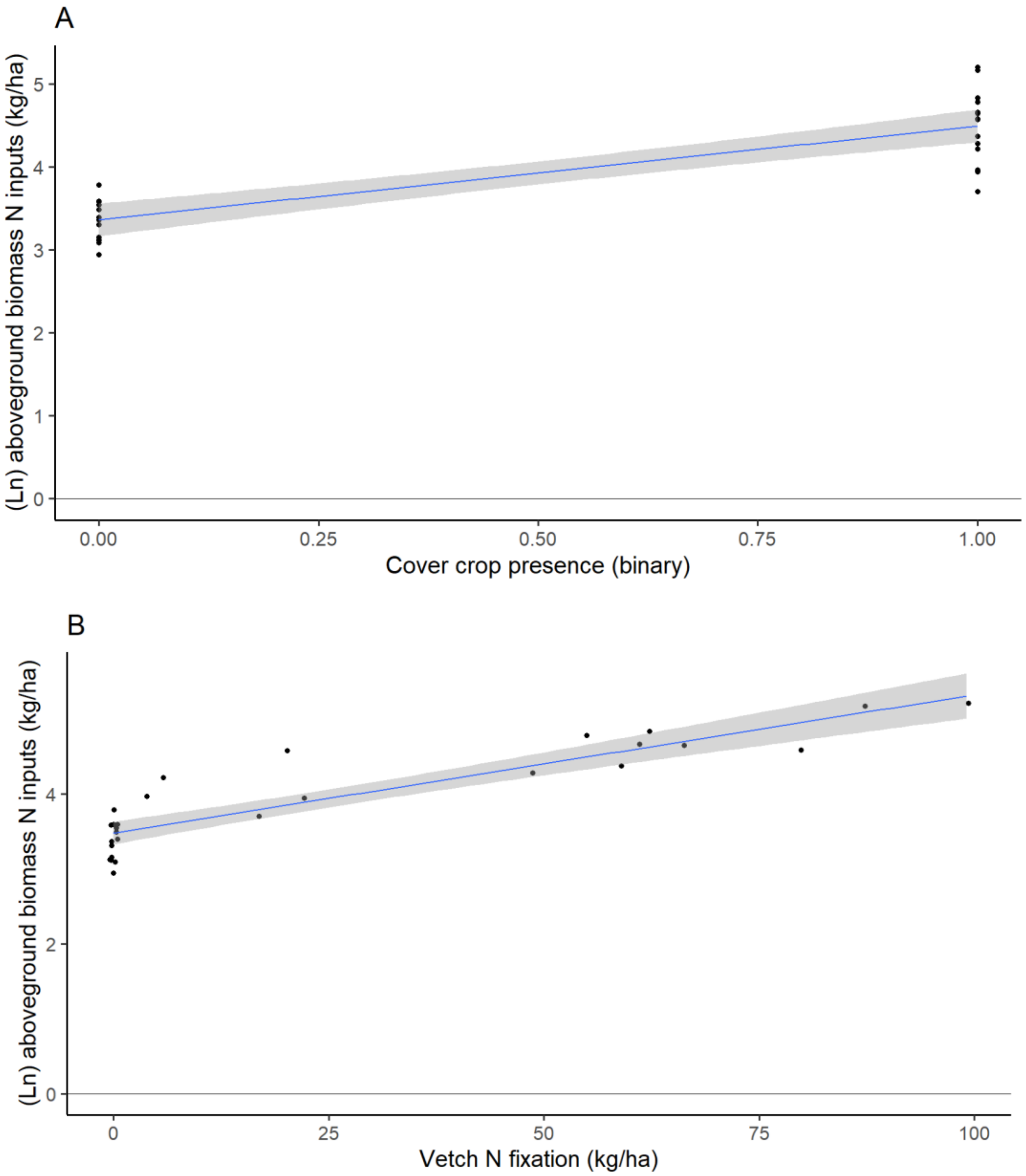


Figure A2.8. Partial regression plots for Model 3 in the full SEM (2019 data), showing relationships between **(A)** cover crop presence and **(B)** total vetch nitrogen (N) fixation in the cover crop mixture and natural log-transformed N inputs from cover crop and weedy fallow aboveground biomass. Weedy fallow plots were assigned a vetch N fixation value of zero. Gray shading indicates standard error. Regression statistics are available in Table A2.3.

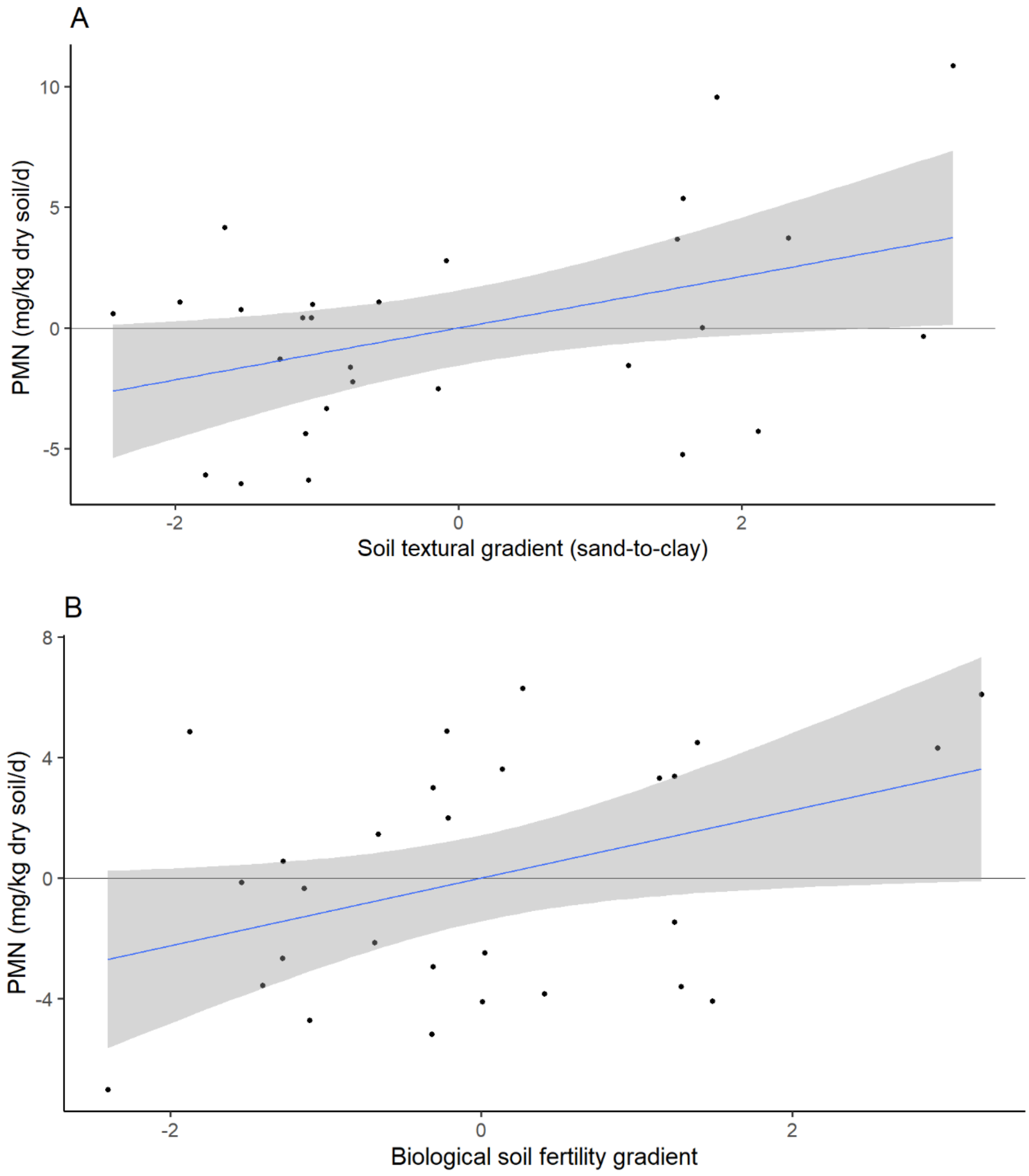


Figure A2.9. Partial regression plots for Model 4 in the full SEM (2019). (**A)** and **(B)** show predictors of potentially mineralizable nitrogen (PMN) in soil from experimental fields, as determined using a 14-day aerobic incubation. Each partial plot represents the variation in PMN explained by one predictor after accounting for all others. Gray shading represents standard error. Regression statistics are available in Table A2.3.

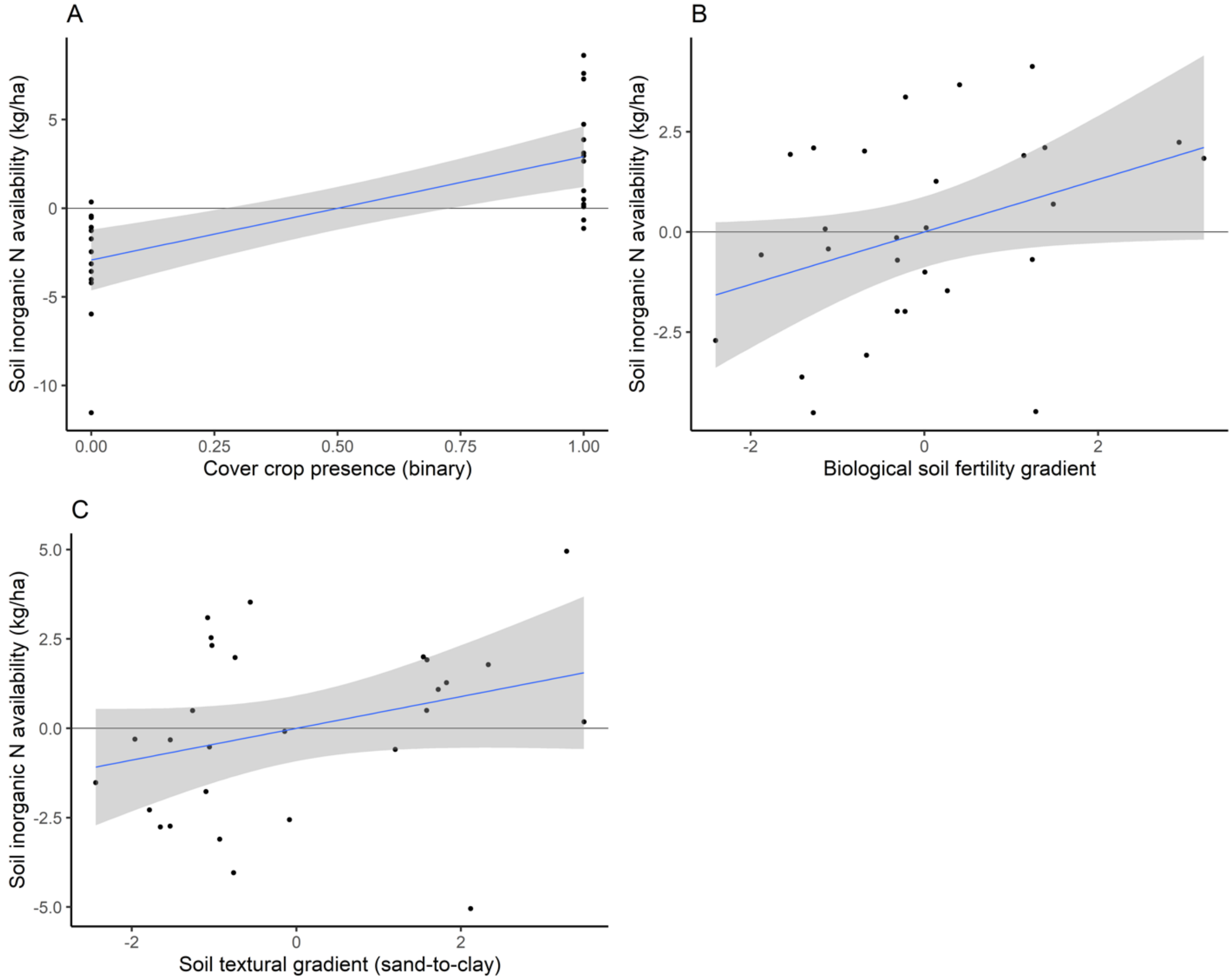


Figure A2.10. Partial regression plots for Model 5 in full SEM (2019). **A, B, and C** show predictors of soil inorganic nitrogen (N) availability following cover crop incorporation; each partial plot represents the variation in inorganic N explained by one predictor after accounting for all others. Gray shading represents standard error. Regression statistics are available in Table A2.3.

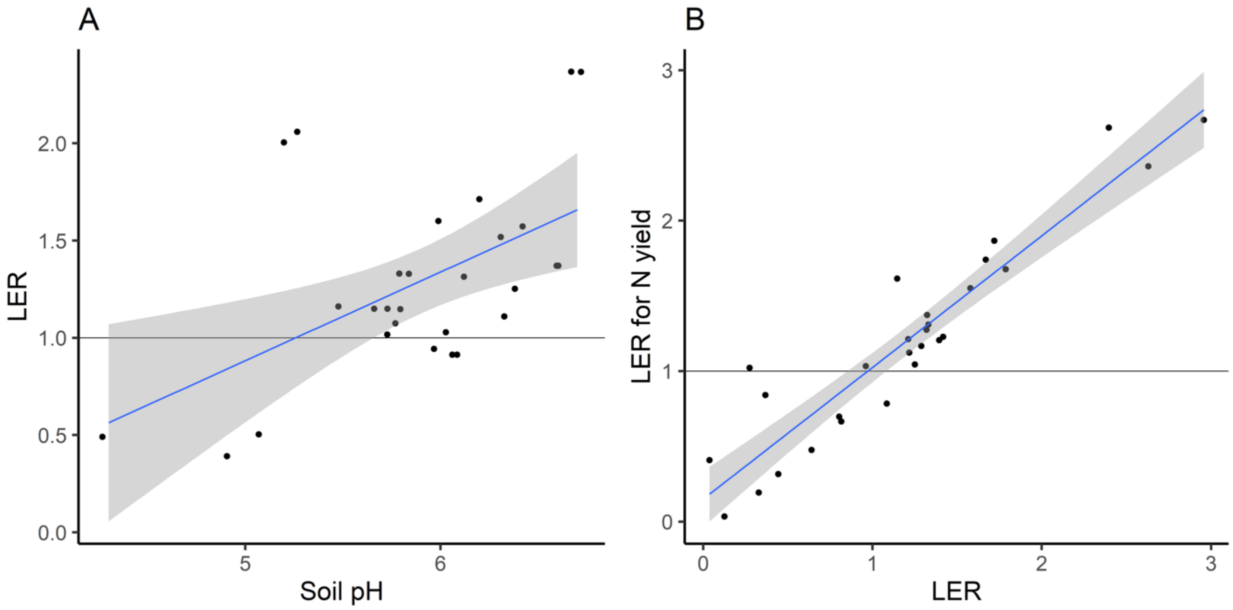
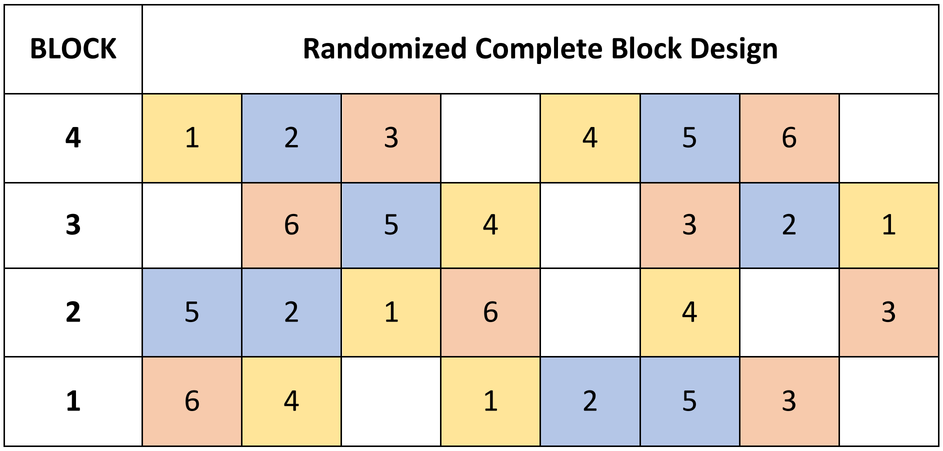


Figure A2.11. Fitted linear regression plots for Models 6 and 7 in the full SEM (2019). (**A)** Fitted linear relationship between soil pH and the Land Equivalent Ratio (LER). **(B)** Fitted linear relationship between LER and the LER for nitrogen yield (LER_N). Gray shading represents standard error. Regression statistics can be found in Table A2.3.

Appendix 3: Supplemental Material for Chapter 4

A3.1 Supplemental methods

Experimental design



BLOCK	Randomized Complete Block Design							
4	1	2	3		4	5	6	
3		6	5	4		3	2	1
2	5	2	1	6		4		3
1	6	4		1	2	5	3	

Figure A3.1. Experimental design with two factors (cover crop and intercrop) and six treatments representing differential factorial combinations, listed in Table A3.1. No-vegetable control plots were included in the experimental design (white squares), but we do not include results from treatments without vegetable crops in the present study.

Table A3.1. Experimental treatments in our study.

Treatment	Treatment name	Cover crop phase	Intercrop phase	Vegetable crops
1	Cover crop + intercrop	Oat-vetch cover crop	Intercrop	Snow pea + cucumber
2	Cover crop	Oat-vetch cover crop	Monocrop	Snow pea
3	Cover crop	Oat-vetch cover crop	Monocrop	Cucumber
4	Intercrop	Weedy fallow	Intercrop	Snow pea + cucumber
5	Control	Weedy fallow	Monocrop	Snow pea
6	Control	Weedy fallow	Monocrop	Cucumber

Soil analyses

Standard Brazilian protocols were used for baseline soil characterization (EMBRAPA, 1997). We extracted Mg, Ca, Al, and Mn in 1M KCl and diluted with 1% HCl prior to analysis on an atomic absorption spectrometer (Analytik Jena contrAA-700). We extracted P and K macronutrients with a Mehlich-1 solution and determined by flame emission spectrophotometry and photocolorimetry (Digimed DM-63). Micronutrients Fe, Zn, and Cu were extracted with a Mehlich-1 and analyzed with an atomic absorption spectrometer.

A3.2 Detailed soil and crop summary tables

Table A3.2. Baseline physical, chemical, and biological soil properties (sampled to 20 cm depth) by block, measured prior to the experiment. PMC: potentially mineralizable carbon.

Baseline soil characteristic	**Experimental Block**				
	1	**2**	**3**	**4**	**Mean (1-4)**
Texture class	sandy loam	sandy loam	sandy loam	sandy loam	sandy loam
Sand (%)	79	80	80	80	80
Clay (%)	14	13	8	7	11
pH (buffered)	5.6	5.5	5.5	5.6	5.6
Bulk density (g/cm^3)	1.18	1.06	1.13	1.07	1.1
Total organic C (Mg/ha)	62.3	58.7	64.1	53.4	59.6
Soil organic C (%)	2.6	2.7	2.9	2.4	2.7
Total N (Mg/ha)	3.9	3.4	3.5	2.8	3.4
Soil total N (%)	0.16	0.16	0.16	0.13	0.15
Soil C:N	16.1	17.1	18.3	18.8	17.6
PMC (μg CO_2-C/g/d)	50.7	48.2	28.3	35.4	40.6
P (kg/ha)	100	65	64	76	76
P (mg/L)	42	30	29	35	34
K (kg/ha)	158	97	108	71	109
K (mg/L)	65	45	49	33	48
Ca (mg/L)	1677	1365	1326	1365	1433
Mg (mg/L)	546	507	468	429	488
Cu (mg/L)	0.8	0.5	0.4	0.4	0.5
Zn (mg/L)	5.8	3.9	4.4	4.5	4.7
Fe (mg/L)	124	118	117	134	123.3
Mn (mg/L)	2.6	2.6	1.9	8.4	3.9
Soil moisture (%)	14	14	14	15	14.3

Table A3.3. Summary table showing cover crop (A) and intercrop (B) outcomes in six diversification treatments across 4 experimental blocks. In (B), results for intercropped treatments show each crop's contribution to the total. Values are treatment means (above) ± italicized standard deviation (below). C: carbon. N: nitrogen. N=4 per treatment and crop type.

A. Cover crop outcomes		**Diversification treatment**					
		Cover + intercrop	**Cover crop**	**Cover crop**	**Intercrop**	**Control**	**Control**
	Crop	Pea + cucumber	Cucumber	Pea	Pea + cucumber	Cucumber	Pea
	Unit						
Cover crop & weeds aboveground biomass	kg/ha	5692	3777	4060	1880	2999	2637
	±	3088	*530*	*834*	*112*	*1794*	*396*
Cover crop & weeds aboveground biomass N	kg/ha	154	82	102	29	41	41
	±	91	*37*	*25*	*6*	*24*	*6*
Cover crop & weeds aboveground biomass C:N	--	18	26	22	28	31	27
	±	2	*11*	*5*	*5*	*2*	*2*
N fixation by vetch	kg/ha	122	59	86	-	-	-
	±	76	*48*	*28*	-	-	-
Soil inorganic N availability at vegetable planting (NO_3^--N + NH_4^+-N)	mg/kg	25	21	33	10	14	15
	±	5	15	14	3	2	3

B. Intercrop outcomes		**Diversification treatment**							
		Cover + intercrop		**Cover crop**	**Cover crop**	**Intercrop**		**Control**	**Control**
	Crop	Cucumber	Pea	Cucumber	Pea	Cucumber	Pea	Cucumber	Pea
	Unit								
Vegetable crop aboveground biomass	kg/ha	104	100	249	254	72	134	114	247
	±	70	17	163	86	37	27	44	15
Vegetable crop aboveground biomass N	kg N/ha	3.3	1.7	5.5	3.8	1.7	2.1	2.4	3.8
	±	*0.6*	*0.1*	*3.4*	*1.2*	*0.9*	*0.5*	*1.0*	*0.4*
Vegetable crop aboveground biomass C:N	--	14.8	28.4	16.8	30.0	16.2	29.6	17.9	29.7
	±	*1.6*	*2.4*	*1.1*	*2.7*	*2.2*	*2.3*	*1.0*	*2.5*
Yield	g/plant	346	13	211	20	182	19	82	16
	±	*109*	*2*	*170*	*7*	*122*	*4*	*22*	*4*
Mean weight per fruit (yield)	g	76.9	3.9	62.7	4.7	56.5	4.8	40.7	4.5
	±	*54.9*	*1.3*	*38.8*	*0.9*	*33.1*	*0.6*	*17.9*	*0.6*
Total protein yield	kg/ha	11.5	10.1	13.3	29.0	5.4	13.6	4.8	22.1
	±	*4.9*	*1.7*	*10.2*	*8.4*	*3.4*	*2.9*	*1.5*	*5.2*
Total mineral yield	kg/ha	5.1	0.8	6.0	2.4	2.6	1.2	2.3	1.9
	±	*1.7*	*0.1*	*4.4*	*0.9*	*1.8*	*0.2*	*0.8*	*0.4*
Total nutrient yield	kg/ha	16.6	10.9	19.3	31.4	8.0	14.9	7.1	24.0
	±	*6.4*	*1.7*	*14.2*	*9.3*	*5.2*	*3.1*	*2.2*	*5.5*

A3.3 Detailed crop nutrient outcomes

Comparing nutrient content (g/100 g fresh weight) and nutrient concentration (% dry weight) results

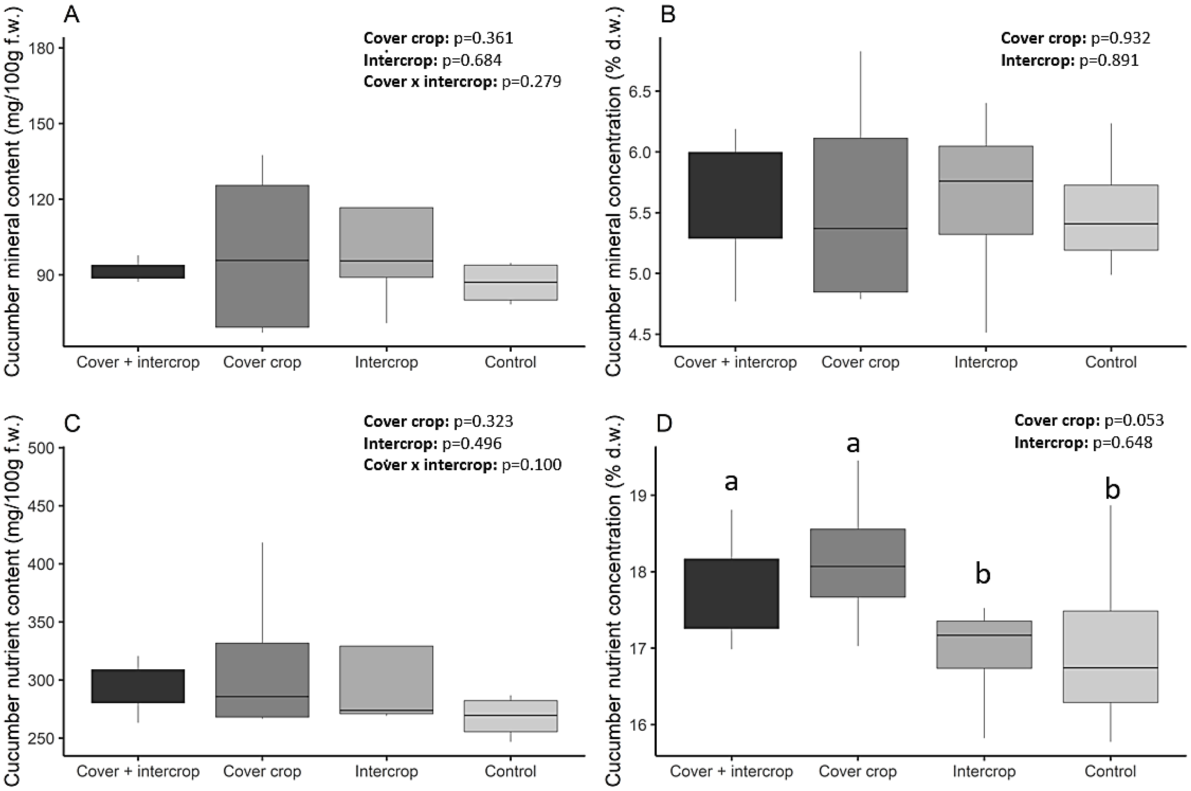


Figure A3.2. Cucumber nutrient content and concentration measured in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control. Minerals included P, K, Ca, Mg, Fe, and Zn. Nutrient yield and content included minerals and protein. Treatments that share a letter are not significantly different at p=0.05 (Tukey's). ANOVA model p-values by treatment are displayed in the top right corner of each panel. F.W.: fresh weight. D.W.: dry weight.

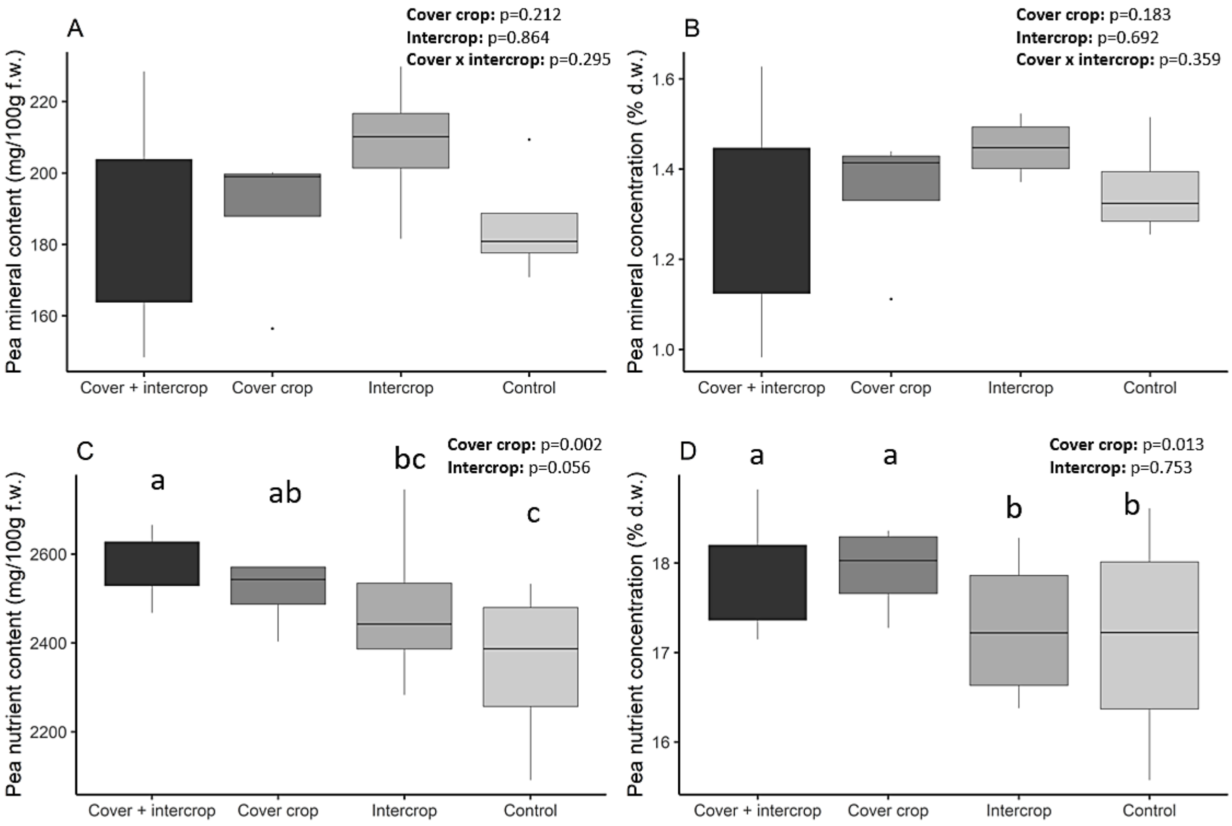


Figure A3.3. Snow pea nutrient content and concentration measured in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control. Minerals included P, K, Ca, Mg, Fe, and Zn. Nutrient yield and content included minerals and protein. Treatments that share a letter are not significantly different at $p<0.05$ (Tukey's). ANOVA model p-values by treatment are displayed in the top right corner of each panel. F.W.: fresh weight. D.W.: dry weight.

Individual nutrient yields, contents, and concentrations by crop type

Cucumber

Table A3.4. Cucumber mixed-effects ANOVA results for total mineral and nutrient concentration, content, and yield. Wald's χ^2 was used to assess statistical significance. Sig. = treatment significance. ~p<0.10, *p<0.05, **p<0.01. Models were additive (if interaction was not significant) or interactive, with cover crop and intercrop treatments as fixed effects, a cover crop by intercrop interaction, and experimental block as a random effect.

Outcome	Unit (outcome)	Treat-ment	Wald's χ^2	Model DF	Model P-value	Sig.	Marginal R^2	Conditional R^2
Total mineral concentration	% dry wt	Cover crop	0.01	1	0.93		0.002	0.002
Total mineral concentration	% dry wt	Intercrop	0.02	1	0.89		0.002	0.002
Total mineral concentration	% dry wt	Cover crop x intercrop	-	-	-		-	-
Total mineral content	g / 100 g fresh wt	Cover crop	0.83	1	0.36		0.08	0.24
Total mineral content	g / 100 g fresh wt	Intercrop	0.17	1	0.68		0.08	0.24
Total mineral content	g / 100 g fresh wt	Cover crop x intercrop	1.17	1	0.28		0.08	0.24
Total mineral yield	g / plant, fresh wt	Cover crop	7.13	1	0.01	**	0.42	0.48
Total mineral yield	g / plant, fresh wt	Intercrop	4.98	1	0.03	*	0.42	0.48
Total mineral yield	g / plant, fresh wt	Cover crop x intercrop	-	-	-		-	-
Total nutrient concentration	% dry wt	Cover crop	3.74	1	0.05	~	0.23	0.23
Total nutrient concentration	% dry wt	Intercrop	0.21	1	0.65		0.23	0.23
Total nutrient concentration	% dry wt	Cover crop x intercrop	-	-	-		-	-
Total nutrient content	g / 100 g fresh wt	Cover crop	0.98	1	0.32		0.11	0.51
Total nutrient content	g / 100 g fresh wt	Intercrop	0.46	1	0.50		0.11	0.51
Total nutrient content	g / 100 g fresh wt	Cover crop x intercrop	2.51	1	0.10	~	0.11	0.51
Total nutrient yield	g / plant, fresh wt	Cover crop	7.77	1	0.01	**	0.43	0.49

Total nutrient yield	g / plant, fresh wt	Intercrop	4.71	1	0.03	*	0.43	0.49
Total nutrient yield	g / plant, fresh wt	Cover crop x intercrop	-	-	-		-	-
Yield	g / plant, fresh wt	Cover crop	8.85	1	0.00	**	0.45	0.54
Yield	g / plant, fresh wt	Intercrop	5.62	1	0.02	*	0.45	0.54
Yield	g / plant, fresh wt	Cover crop x intercrop	-	-	-		-	-
Water content	g / 100 g fresh wt	Cover crop	4.79	1	0.03	*	0.12	0.63
Water content	g / 100 g fresh wt	Intercrop	4.10	1	0.04	*	0.12	0.63
Water content	g / 100 g fresh wt	Cover crop x intercrop	6.27	1	0.01	*	0.12	0.63

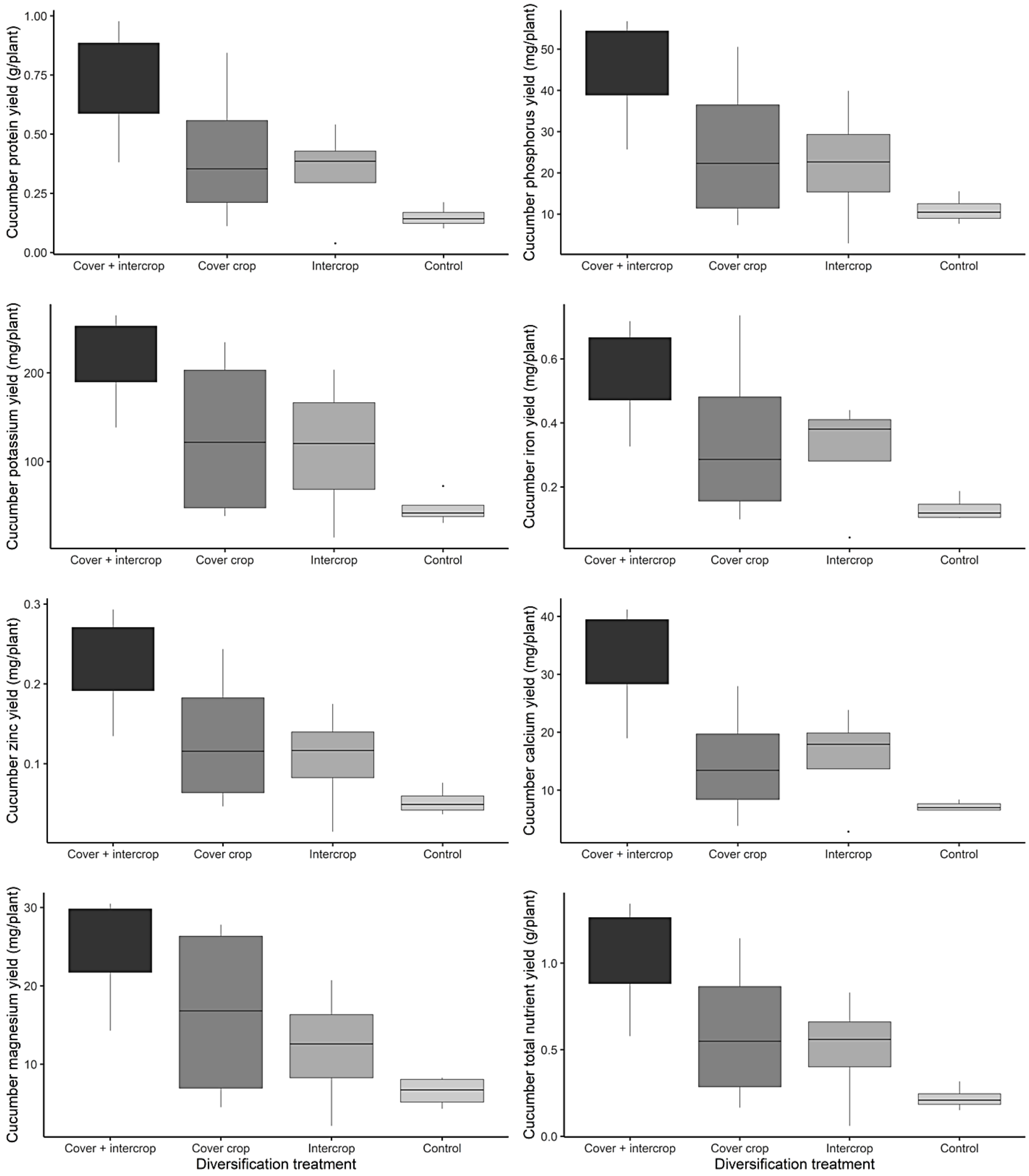


Figure A3.4. Cucumber individual nutrient yields (g or mg/plant) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

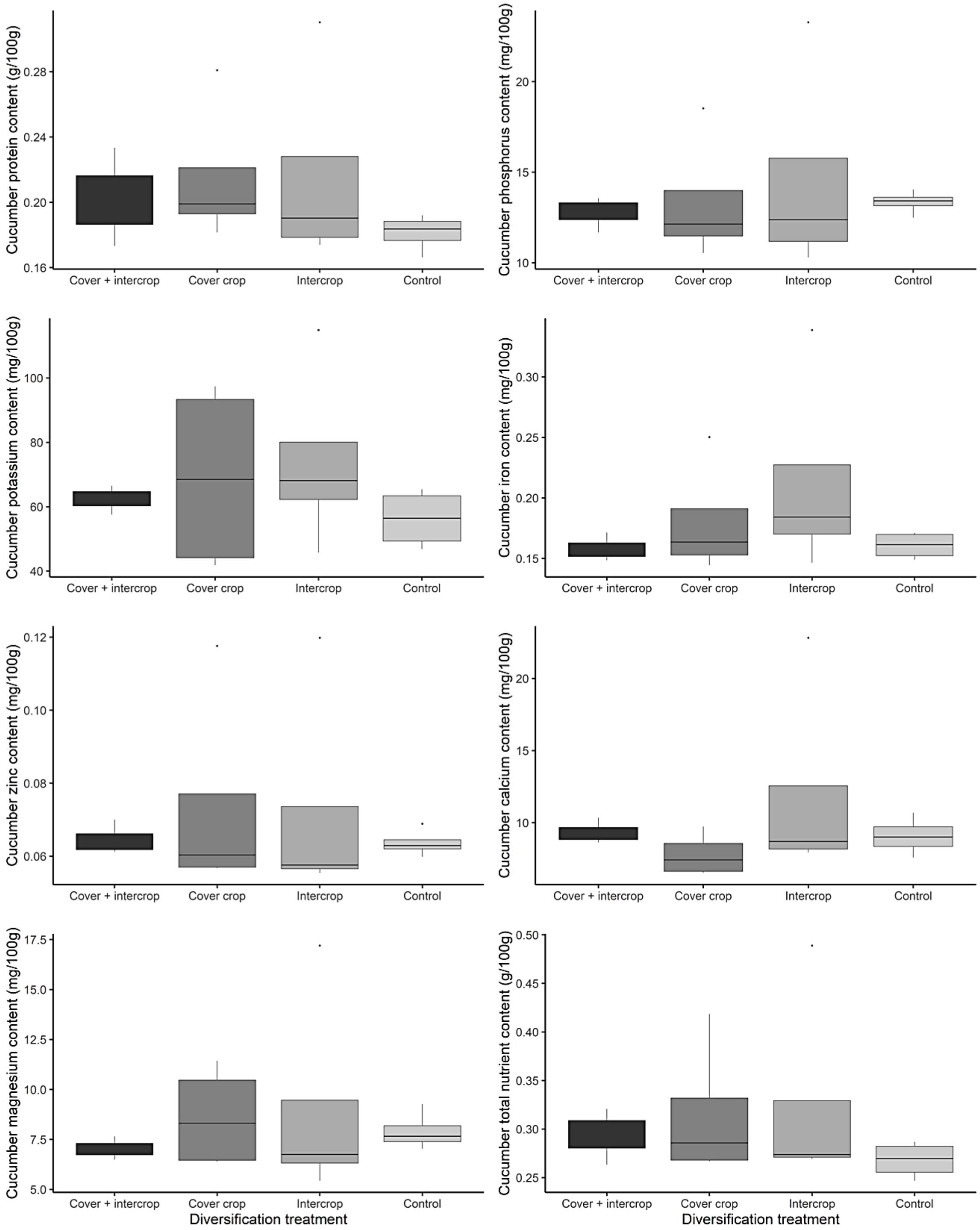


Figure A3.5. Cucumber nutrient content (g or mg/100 g fresh weight) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

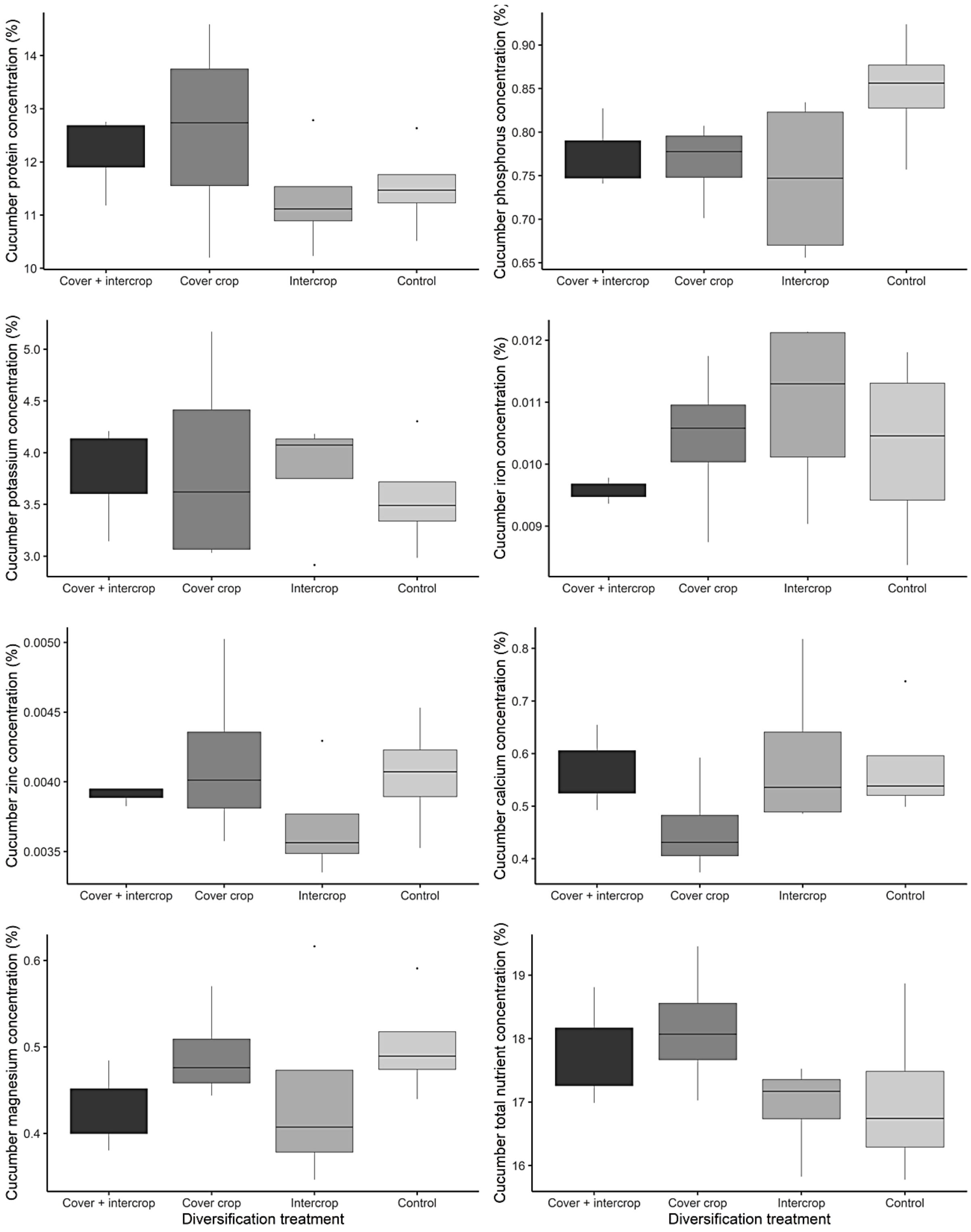


Figure A3.6. Cucumber nutrient concentrations (% dry weight) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

Snow pea

Table A3.5. Snow pea mixed-effects ANOVA results for total mineral and nutrient concentration, content, and yield. Wald's χ^2 was used to assess statistical significance. Sig. = treatment significance. ~p<0.10, *p<0.05, **p<0.01. Models were additive (if interaction was not significant) or interactive, with cover crop and intercrop treatments as fixed effects, a cover crop by intercrop interaction, and experimental block as a random effect.

Outcome	Unit (outcome)	Treat-ment	Wald's X^2	Model DF	Model P-value	Sig.	Marginal R^2	Condition-al R^2
Total mineral concentration	% dry wt	Cover crop	1.77	1	0.18		0.09	0.39
Total mineral concentration	% dry wt	Intercrop	0.16	1	0.69		0.09	0.39
Total mineral concentration	% dry wt	Cover crop x intercrop	0.84	1	0.36		0.09	0.39
Total mineral content	g / 100 g fresh wt	Cover crop	1.56	1	0.21		0.14	0.22
Total mineral content	g / 100 g fresh wt	Intercrop	0.03	1	0.86		0.14	0.22
Total mineral content	g / 100 g fresh wt	Cover crop x intercrop	1.10	1	0.29		0.14	0.22
Total mineral yield	g / plant, fresh wt	Cover crop	4.79	1	0.03	*	0.31	0.34
Total mineral yield	g / plant, fresh wt	Intercrop	4.10	1	0.04	*	0.31	0.34
Total mineral yield	g / plant, fresh wt	Cover crop x intercrop	6.27	1	0.01	*	0.31	0.34
Total nutrient concentration	% dry wt	Cover crop	6.18	1	0.01	*	0.15	0.68
Total nutrient concentration	% dry wt	Intercrop	0.10	1	0.75		0.15	0.68
Total nutrient concentration	% dry wt	Cover crop x intercrop	-	-	-		-	-
Total nutrient content	g / 100 g fresh wt	Cover crop	9.22	1	0.00	**	0.24	0.75
Total nutrient content	g / 100 g fresh wt	Intercrop	4.94	1	0.03	*	0.24	0.75
Total nutrient content	g / 100 g fresh wt	Cover crop x intercrop	-	-	-		-	-
Total nutrient yield	g / plant, fresh wt	Cover crop	2.46	1	0.12		0.27	0.27
Total nutrient yield	g / plant, fresh wt	Intercrop	3.63	1	0.06	~	0.27	0.27

Total nutrient yield	g / plant, fresh wt	Cover crop x intercrop	4.94	1	0.03	*	0.27	0.27
Yield	g / plant, fresh wt	Cover crop	3.45	1	0.06	~	0.28	0.28
Yield	g / plant, fresh wt	Intercrop	4.50	1	0.03	*	0.28	0.28
Yield	g / plant, fresh wt	Cover crop x intercrop	4.97	1	0.03	*	0.28	0.28
Water content	g / 100 g fresh wt	Cover crop	0.02	1	0.88		0.21	0.27
Water content	g / 100 g fresh wt	Intercrop	0.81	1	0.37		0.21	0.27
Water content	g / 100 g fresh wt	Cover crop x intercrop	0.23	1	0.63		0.21	0.27

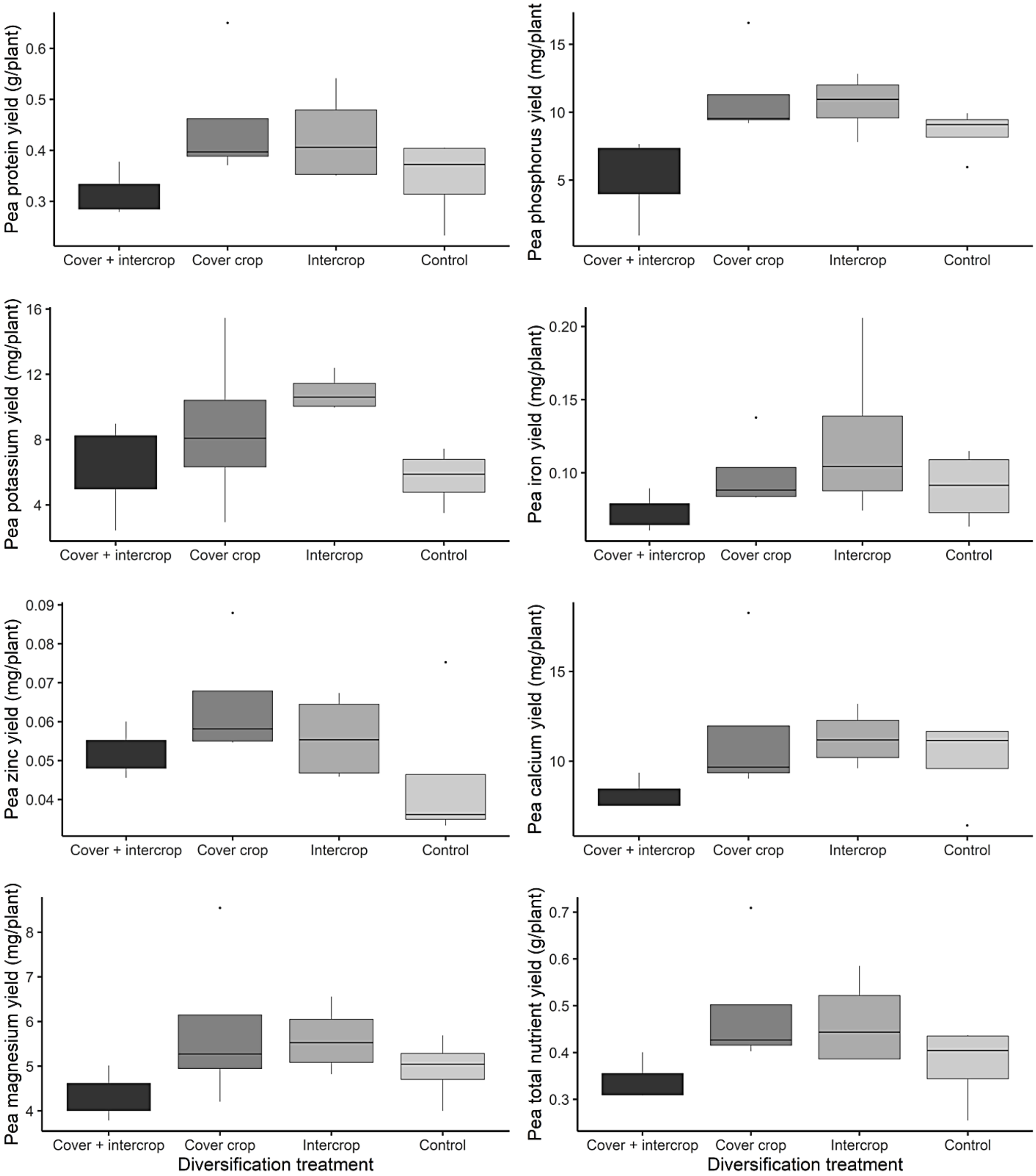


Figure A3.7. Pea individual nutrient yields (g or mg/plant) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

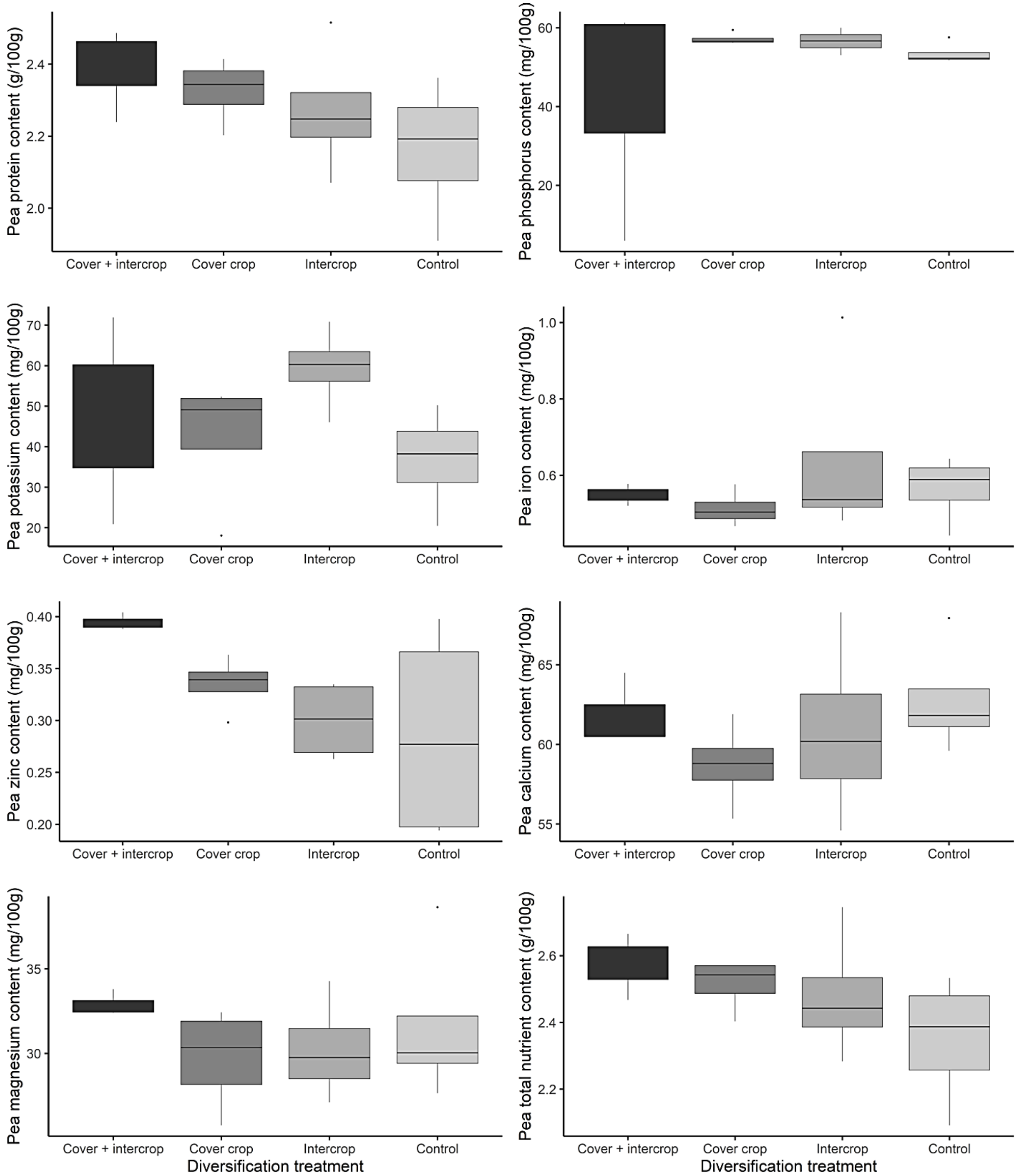


Figure A3.8. Snow pea nutrient content (mg or g/100 g fresh weight) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

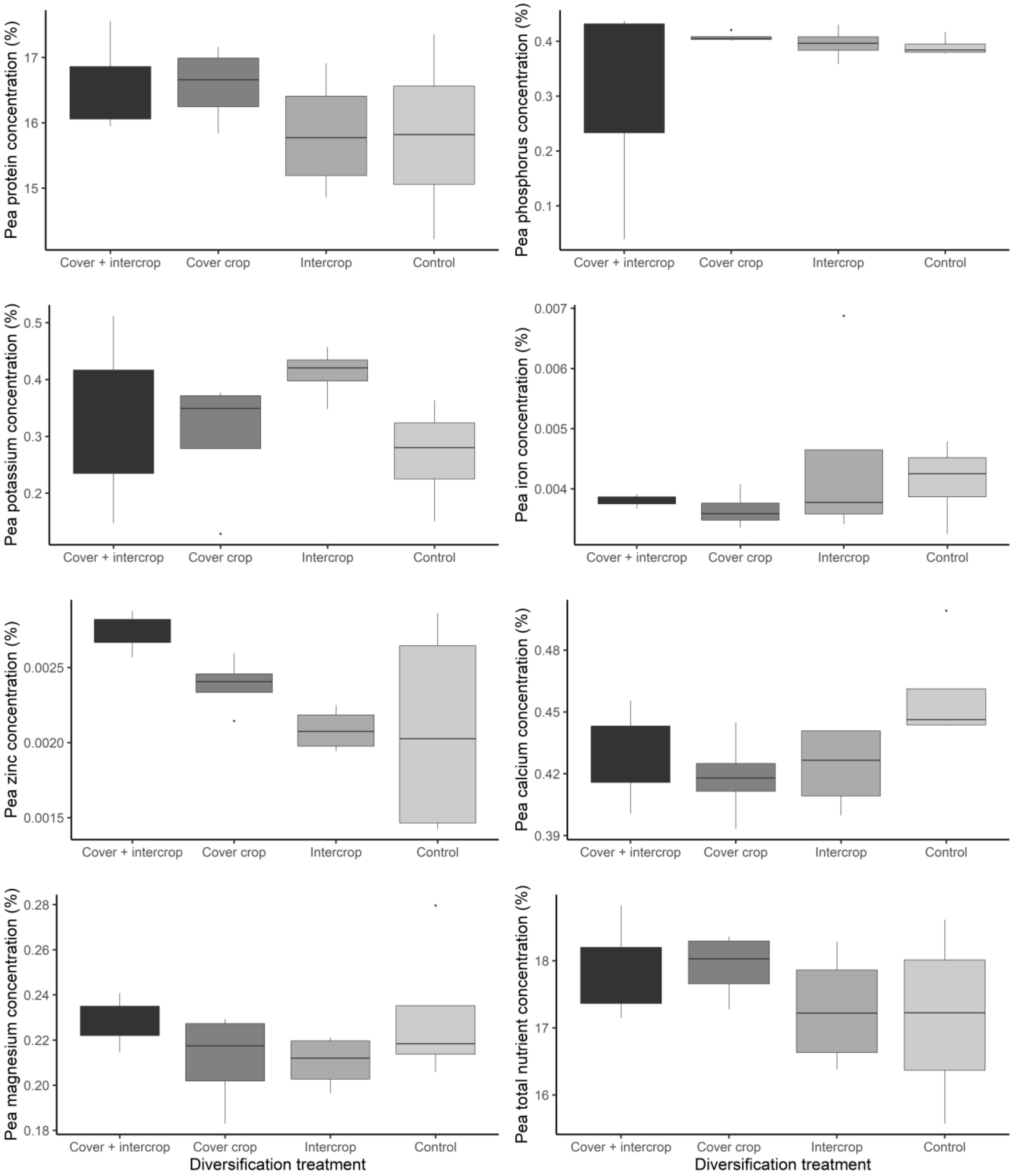


Figure A3.9. Snow pea nutrient concentrations (% dry weight) in four treatments: cover crop + intercrop (combined diversification), cover crop, intercrop, and control.

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