Cover Crops, Carbon Dynamics and the Ecological Intensification of Vineyards – A View From Under the Vine.

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Preface

Before we move forward through lexical thickets, of cover crops, carbon and p-values, I want to take a moment – *just briefly* – to discuss some of the fundamental philosophies that have attached themselves to my thoughts and actions while completing this PhD. I started studying science six years ago, having acquired degrees in Arts and International Studies previously. Ironically, I decided to venture into science partly to become a better writer – less floral and long-winded (science doesn't allow that). I think I succeeded, though I still try to slip in some off-beat esoterica every now and then. Sometimes it passes the review process, sometimes not… I want to share a few of these thoughts with you now. Perhaps because I'm feeling sombre at the end of this process (or is it the beginning?) or perhaps because sometimes scientific theses need a little bit of esoterica layered between the numbers and figures.

I'm something of a fundamentalist. That is, when confronted with a problem, puzzle or project, I like to wind it back to its origin, to understand its *essence*. I think this came from a childhood spent asking *why? Yeah, but why? Yeah, but why?* – I must have been a pain. Children ask these questions and we often see it as impudence or cheek but I've come to regard it more as an inquisition into the layers of complexity that surround so much of life's oddities. Physicists spend much of their time undressing the fundamental nature of… nature. Stripping back atoms and subatomic particles to reveal their most basal forms – all the better for understanding us and our universe. I find it a necessary act but also often anxiety-inducing as I wait to see what new truth lurks around the corner.

Specificity is expensive. It's also correct. Generality is cheap. It's also lazy and often wrong. I discuss specificity a lot throughout this thesis because it's important for getting it right. Viticulturists and winemakers will discuss *terroir,* the notion that vineyards are all unique – a function of their biotic and abiotic properties. The essence of terroir is specificity – a particular grape varietal planted in a particular location and tended to in a particular way will result in a wine that smells and tastes utterly unique. It is a romantic notion but it is quantifiable and so it has truth in its essence. Selecting which cover crop to plant and how to tend to the vineyard floor is also a tale of specificity, rather than generality and I hope this becomes conveyed throughout this document.

The simplest and most fundamental things in life are often so overlooked that in many cases they require rediscovering. Communication is one of these things. I firmly believe that poor communication is at the heart of failure for so many companies, organisations, institutions and relationships. It seems like a simple one, and it is, but too often I see how easily certain issues or problems could be avoided or resolved if only the human constituents communicated thoughts and feelings with clarity. Indeed, I have been guilty of this sin time and again and I hope I continue to learn. I have been fortunate to work within a lab group that has excellent communication and for this I am grateful.

Unfortunately we live in a world where the vast majority of systemic and institutionalised power rests with men. For me, it's an experiment that we've tried for centuries and it is not altogether working. Power is an interesting concept. I often ruminate that power cannot be ceded, it must be taken. Ceding power is only having the power to cede power, rather than taking it and therefore being truly *powerful*. The most fundamental division in humanity is between *male* and *female*. To only ever experience a world where the male perspective is dominant in institutional governance is wrong, perhaps even *sick*. I write these words on the eve of my twin sons being born and will aim to raise them to understand that balance is essential for a functional society. I hope the fundamental changes are in place to allow the next generation to govern with core philosophies that are rooted in empathy and connectivity – both to one and other and also to our natural environment.

Finally, and with great sincerity, I want to acknowledge the traditional owners of the land upon which I have dug, sieved and analysed these past three years. More than anything, I want to acknowledge the many first nations that exist within Australia and pay respect to their elders and leaders – those who I desperately hope will gain more voice and power to govern this vast and beautiful landmass that has been a cradle of human existence for some 60,000 years or longer. As an ecologist, I can learn much from traditional land management that has been passed down generations. I hope it remains and I hope we can better acknowledge it and be humble enough to learn from it.

Abstract

There is mounting pressure to act more sustainably regarding agricultural land use, with viticulture no exception. How vineyard floors are managed can have broad ramifications, not only for grapevines but for the ecology of the vineyard and, importantly, for the soil beneath it. Traditional practices have often employed herbicides to maintain bare earth alleys, reducing grapevine competition with weeds. Many vineyards throughout the world have adopted some form of alternative management practice, at least in the vineyard mid-row, often revolving around the use of cover crops or a wild sward. The adoption of under-vine (or whole-floor) cover crop use has been somewhat slower, owing to apprehension over the perceived increase in direct competition between cover crops and grapevines for water and essential nutrients. It is hoped that by isolating and quantifying key vineyard floor variables we are better able to understand the influence of under-vine cover crop use and thus more accurately inform vineyard floor management practices.

This thesis explores the theme of ecological intensification *under-vine* and thus how cover crops function as ecosystem service providers to the benefit of under-vine soil. Specifically, the following details experimentation conducted on two vineyards in South Australia, where seven treatments (five cover crop combinations, a straw mulch and herbicide-managed control) were established in 2014. Experiment one sampled soil under-vine and, through a pot-growth trial, aimed to determine whether cover crops increased the arbuscular mycorrhizal (AM) inoculum potential of both a leek trap plant and trial-based cover crops. In this, we were unable to distinguish differences in inoculum potential between different soils and concluded that AM inoculum may be buffered by the presence of other vineyard plants. Experiment two sought to quantify soil organic carbon (SOC) stocks and turnover in two vineyards, to a stratified depth of 0-10 cm and 10-30 cm. Results showed that SOC stocks were up to 23% higher under cover crop-managed treatments than the herbicide control. Experiment three employed a litter bag trial and laboratory incubation experiment to determine whether cover crop residues decompose more readily in their *home* soil, rather than their *away* soil. In this, we were unable to determine significant differences in decomposition in *home* vs *away* soils and concluded that the

heterogeneity and frequent disturbance within vineyards were likely factors in dampening any *home-field* effects with regards to decomposition dynamics.

In each hypothesis and subsequent experimental trial, we attempted to understand the role of under-vine cover crops as providing ecosystem services to the benefit of the vineyard floor. The results suggest that cover crops sown under-vine can provide positive ecosystem services to the vineyard floor. Although we found non-significant results regarding AM inoculum potential and *home-field advantage* decomposition, there were no deleterious results of cover-cropping relative to herbicide use, and further experimentation may reveal significantly beneficial results. At a time when global CO_2 emissions have reached detrimental levels, the planting of bare earth to draw atmospheric carbon into the soil is perhaps one of the most beneficial practices in agroecosystems. Moreover, the co-benefits of increasing SOC in vineyards are quantifiably numerous and are discussed in detail in chapter two.

Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I acknowledge that copyright of published works contained within the thesis resides with the copyright holder(s) of those works.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Joseph Marks

01/11/2022

Acknowledgements

I have a lot to be grateful for and consequently, a lot of people to whom I owe gratitude. First and foremost, I want to acknowledge and pay enormous respect to Timothy Cavagnaro and Thomas Lines. Tim has been an outstanding principal supervisor – a perfect balance of tutor, mentor and colleague. I have, as have many in my position, been prone to mid-PhD “freak-outs”, catastrophising all manner of tiny things. Tim is a person who instils instant calm – “*that’s okay, we can deal with it, it’s no problem*”. Tim goes above and beyond for all his students and I wish every PhD candidate could have a supervisor as diligent and understanding as he is. I have been Tom’s first student and I call him one of the most intelligent and decent people I’ve ever met. Tom has a rare gift of being able to listen, debug and instantly understand the often incomprehensible babble that comes out of my mouth when trying to distil an idea or concept. Like Tim, Tom has offered me incredible mentoring and collegiality throughout my candidature. Above all, Tom has become one of my closest friends – a two-for-one deal with a supervisor. More than anything else, Tim and Tom are outstanding men – something that the world desperately needs more of.

Thank you to Vinay Pagay, who lent his expansive knowledge of viticulture and grapevine physiology to marry with my interest in soil ecology and increase my understanding of the vineyard ecosystem. Chris Penfold was not strictly a supervisor but he was a mentor, collaborator and friend who imparted great wisdom. Chris was the progenitor of the vineyard trial sites back in 2014 and it is a testament to his diligence that I have been able to experiment with them these past years.

The CavLab is an extension of Tim, and within it is an ever-changing group of kind, intelligent and team-oriented people. I acknowledge the teamwork, communication and collegiality of the group, with special thanks to Matthias Salomon, Erinne Sterling, Christina Asanopoulos, Isobel Hume, Hue Ngo, Jade Rose, Cuc Tran, Viran Arachchige and Graeme Anderson. Moreover, I would like to pay respect to the entire Soil Science department. Within these groups are some of the nicest people at the University and I want to particularly acknowledge the mentoring and support of Ron Smernik and Petra Marschner.

To my family and friends. Above all else I want to acknowledge my partner, Georgina who, only the day after this thesis concluded, gave birth to our twin boys, Emile and Luca, and who has shown me nothing but love and support from undergrad six years ago, to the completion of this thesis. She is the kindest and most empathetic human I have ever known and I would not have undertaken any of this without her encouragement. To my mother, Rose, and father, Andrew, thank you for believing in me and providing me with every opportunity and advantage in life – I am extremely privileged. Thank you to my brothers, Jake and Harry and my best friend in the world, Mark. I also want to thank Tamara Tiller, manager at Penfolds Magill Estate cellar door, my friend and former boss. I spent five years at Penfolds and it was one of the most pivotal learning experiences of my life – vastly increasing my interest and knowledge in wine and viticulture and developing my confidence in public speaking. I am grateful to her for always taking my studies into consideration and providing me with just the right amount of shifts.

Finally and sincerely, I want to thank both the University of Adelaide and Wine Australia for their support – both academic and financial. To Wine Australia, for awarding me the *Tony Jordan OAM Award* and providing an incredibly generous top-up scholarship, which has allowed me to focus entirely on my studies, thank you so very much. Within the team at Wine Australia I particularly want to thank Alex Sas, Jo Hargreaves, Paul Smith, Liz Waters and Belinda Bramley. Additionally, I would also like to thank Stephanie Watts-Fawkes and Lynne Macdonald (CSIRO) for their support and advice and Robyn Dixon (AWRI) for generously inviting me to speak on a number of occasions.

This process – so often fraught with mental anguish – has been as smooth and functional as I could have hoped and this is all thanks to the excellent teamwork, communication and collegial environment fostered within the soils groups and the Waite Research Institute.

Abbreviations

AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
C	Carbon
DOC	Dissolved organic carbon
F+C	Fescue + clover
HC	Herbicide control
HSD	Honestly significant difference
KC	Kasbah cocksfoot
M+M	Medic + medic
M+R	Medic + ryegrass
N	Nitrogen
OC	Organic carbon
RCBD	Randomised complete block design
RO	Reverse osmosis
SOC	Soil organic carbon
SOM	Soil organic matter
TM	Triticale mulch
TN	Total nitrogen
TOC	Total organic carbon
WG	Wallaby grass

Chapter One: Introduction

1 Thesis Overview

The following is a thesis by publication (or a hybrid thereof) and has been compiled in accordance with the University of Adelaide guidelines for the submission of a thesis for the degree of Doctor of Philosophy. It is a compilation of three primary research manuscripts (one published) and one review article. The published manuscript has been included in the format in which it was published in the journal *Science of the Total Environment.* The unpublished manuscripts have been formatted to the style of those journals to which they have been submitted for publication. The thesis has been numbered continuously to align with the table of contents and concludes with a general discussion, synthesising each chapter both separately and in the context of the overarching theme of the thesis.

1.1.1 *Chapter structure and approach*

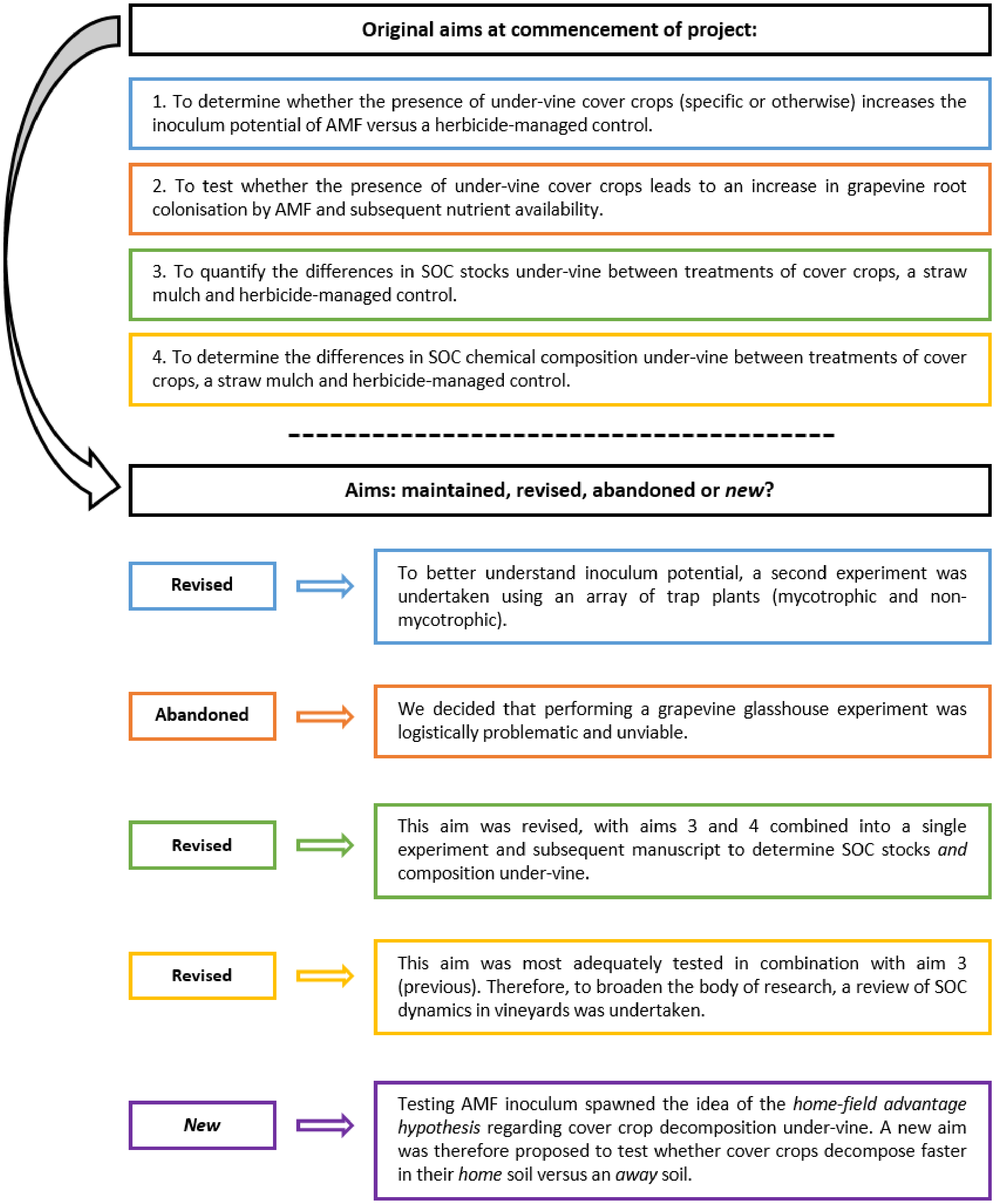
The body of this thesis has been divided into two sections – Part A (Cover Crops and Carbon Dynamics Under-Vine) and Part B (Cover Crops, AMF Inoculum and Decomposition Dynamics Under-Vine).

Part A comprises chapters two and three and focuses on under-vine management practice (particularly cover crop use) and soil organic carbon (SOC) dynamics (stocks, composition and turnover). Chapter two is a review paper focusing on the role of alternative under-vine management practices to increase SOC under-vine (and mid-row). As a comprehensive review of vineyard floor management and SOC dynamics, it will serve as both a core chapter and a literature review for Part A of the thesis. As a scientific review, it has been submitted to the *Australian Journal of Grape and Wine Research*. Chapter three is a primary research paper on under-vine management practices and SOC stocks and turnover in two South Australian vineyards. This is a published body of work and, as such, has been inserted into the thesis document in the format in which it was published within the journal *Science of the Total Environment.*

Part B comprises chapters four, five and six and focuses on the ecological intensification of the vineyard under-vine. Chapter four is a literature review (not intended for publication) and aims to introduce cover crops and their various ecophysiological properties with regards to those topics covered in Part B: arbuscular mycorrhizal (AM) inoculum potential (chapter five) and cover crops and decomposition under-vine (chapter six). Chapter five is a primary body of research on under-vine management and AM inoculum potential and is included as an unpublished manuscript. Chapter six is a primary body of research on under-vine cover crop decomposition and is again included as an unpublished manuscript. Both manuscripts have been submitted for review.

The thesis concludes with chapter seven – a general discussion. This will serve to synthesise all bodies of research, linking together various results and conclusions under several central themes. This will also serve as a review of each body of research separately, summarising key findings and framing them in the context of ecological intensification and vineyard ecosystem services. This chapter will conclude with *future studies* – a brief section that highlights further research that may be undertaken to increase knowledge and understanding around those hypotheses explored within this thesis.

1.1.2 *Research aims: from past to present…*



Original aims at commencement of project:

1. To determine whether the presence of under-vine cover crops (specific or otherwise) increases the inoculum potential of AMF versus a herbicide-managed control.

2. To test whether the presence of under-vine cover crops leads to an increase in grapevine root colonisation by AMF and subsequent nutrient availability.

3. To quantify the differences in SOC stocks under-vine between treatments of cover crops, a straw mulch and herbicide-managed control.

4. To determine the differences in SOC chemical composition under-vine between treatments of cover crops, a straw mulch and herbicide-managed control.

Aims: maintained, revised, abandoned or *new*?

Revised	To better understand inoculum potential, a second experiment was undertaken using an array of trap plants (mycotrophic and non-mycotrophic).
Abandoned	We decided that performing a grapevine glasshouse experiment was logistically problematic and unviable.
Revised	This aim was revised, with aims 3 and 4 combined into a single experiment and subsequent manuscript to determine SOC stocks *and* composition under-vine.
Revised	This aim was most adequately tested in combination with aim 3 (previous). Therefore, to broaden the body of research, a review of SOC dynamics in vineyards was undertaken.
New	Testing AMF inoculum spawned the idea of the *home-field advantage hypothesis* regarding cover crop decomposition under-vine. A new aim was therefore proposed to test whether cover crops decompose faster in their *home* soil versus an *away* soil.

Part A:

Cover Crops and Carbon Dynamics Under-Vine

Chapter Two: A Review of Cover Crops and Soil Organic Carbon in Vineyards.

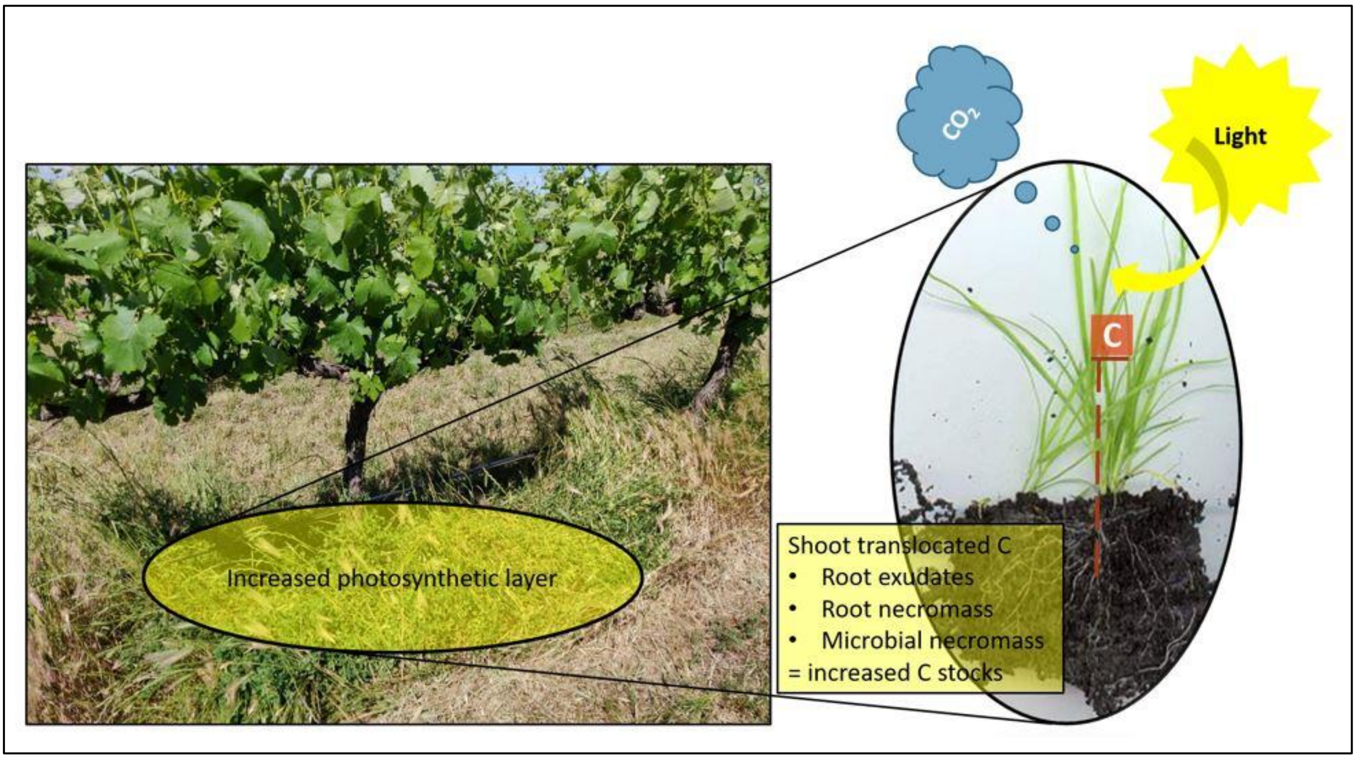
2 From the ground up: A review of cover crops and alternative vineyard floor management practices to build soil organic carbon

I like a review paper – they're a great jumping-off point for exploring a research topic.

Soil organic carbon is such an interesting area of research – both important and topical.

I hope other researchers find this review as useful as I have found others.

Moreover, I hope this area of research helps us to think more sustainably regarding soil health.



Statement of Authorship

Title of Paper	From the ground up: A review of cover crops and alternative vineyard floor management practices to build soil organic carbon.
Publication Status	☐ Published ☐ Accepted for Publication ✓ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	This manuscript has been submitted to the Australian Journal of Grape and Wine Research.

Principal Author

Name of Principal Author (Candidate)	Joseph Marks		
Contribution to the Paper	Conceptualisation, data retrieval, knowledge, drafting & editing.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/04/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Thomas Lines		
Contribution to the Paper	Conceptualisation, knowledge, drafting & editing.		
Signature		Date	21/04/2022

Name of Co-Author	Professor Timothy Cavagnaro		
Contribution to the Paper	Conceptualisation, knowledge, drafting & editing.		
Signature		Date	2nd May, 2022

Abstract

The use of cover crops and alternative vineyard floor management practices are under increasing focus, as herbicide use and conventional tillage have fallen out of favour due to greater regulatory pressure and counter evidence respectively. Moreover, the quantifiable evidence for these transitions in management is growing, with a number of mid-row and under-vine studies reporting the positive influences of cover crops, mulch, compost and biochar. One such influence pertains to the active increase and retention of soil organic carbon (SOC) in vineyard systems. Enhancing SOC dynamics in vineyards (and like agroecosystems) has numerous benefits to soil, both physically, chemically and biologically. Here we examine several notable studies spread across several countries, with contrasting climate and terroirs. Treatment-based interventions show that cover crops can increase (or retain) SOC concentrations and stocks by up to 2.4 times as compared with traditional, bare earth (herbicide or tillage) practices. Furthermore, SOC dynamics stretch beyond concentrations and stocks. Physicochemical composition of SOC changes dramatically with the derivative material, whether that be cover crops, mulch, manure or biochar. Microbial activity can be used as a proxy for carbon turnover, with more labile SOC fractions undergoing rapid turnover, while more recalcitrant materials take longer to decompose. Methodological approaches are also numerous and require logistical specificity, especially in approaches to sample depth, most notably under-vine. This review suggests that cover crops are a beneficial alternative to traditional herbicide use when it comes to building SOC. Moreover, cover crops can provide valuable co-benefits to vineyard floor dynamics, contributing to erosion-prevention, infiltration rates and plant available water content, among much else.

Glossary of terms

Bulk density: The mass of soil within a known volume (e.g. soil core) and is often presented in grams of soil per centimetre squared.

Carbon stock: The amount of C in a known volume of soil. Carbon stocks are product of both the SOC concentration and the bulk density of the soil in which the concentration is measured and is typically presented as tonnes of C per hectare of soil.

Cover crop: A crop planted next to, underneath or in rotation with the focal (cash) crop. Typically, it is not consumed but operates as an ecosystem service provider – enhancing soil fertility and physicochemical properties.

Dissolved organic carbon (DOC): Recognised as a distinct SOC fraction. The most soluble form of SOC and the most microbially-available. It is characterised as being able to pass through filter membranes of between 0.45 – 0.75 µm (Bird *et al.* 2015).

Labile organic C: At the chemical level, labile molecules are those whose bonds are most easily broken and separated into constituent molecules. Labile pools of SOC are those that are more easily decomposed by soil microbial communities (Lal *et al.* 1997).

Particulate organic carbon (POC): Another distinct SOC pool, comprised of larger particles of organic matter. Can become bound to micro-aggregates, increasing its lifespan/stability, reducing mobility, and microbial availability (Witzgall *et al.* 2021).

Recalcitrant organic C: The opposite of labile – recalcitrant or resistant organic C is comprised of molecules whose bonds are less easily broken. Lability and recalcitrance form a spectrum of SOC pools and are key determinants in SOC turnover and residence time (Lal *et al.* 1997).

Soil organic carbon (SOC): The component of soil that is comprised of organically-derived carbon, which can be further divided into pools (or sub-categories), based on physical or chemical composition (see above) (Brady 2008).

2.1 Introduction

Vineyard floor management has undergone something of a renaissance in recent years. In many of the world's wine regions it has long been regarded with fastidious and noble import (Pardini *et al.* 2002). The renaissance – or revision – therefore, refers to the application of scientific inquiry and methodological approaches to the quantification and understanding of different management practices and their multifarious influences on soil-grapevine interactions (Delpuech & Metay

2018; Steenwerth & Belina 2008). In vineyard systems, cover crops are often sown between rows of vines (mid-row cropping) (Campos *et al.* 2019), with mounting evidence showing numerous benefits (Delpuech & Metay 2018). Indeed, mid-row (and whole floor) cover-cropping has been shown to influence soil-grapevine variables such as nutrient cycling and mobilisation (Guerra & Steenwerth 2012; Klodd *et al.* 2016; Pérez Alvarez *et al.* 2015); water retention; soil stabilisation (Klodd *et al.* 2016; Monteiro & Lopes 2007); weed management (Campos *et al.* 2019); control of vine vegetative growth; (Giese *et al.* 2014; Wheeler *et al.* 2005); increased biodiversity (Guerra & Steenwerth 2012); temperature control (Collins & Penfold 2014) and, last but not least; increased soil organic carbon (see below) (Ball *et al.* 2020; Marks *et al.* 2022; Steenwerth & Belina 2008). Where mid-row cover crop use has become widely adopted, under-vine cover-cropping has been met with a more tentative approach, owing to a perceived increase in interspecific competition between grapevine and cover crop (Marks *et al.* 2022). We may begin to see a shift in the once-negative perception of under-vine cover crop use, with mounting research suggesting neutral or positive influences to vineyard and grapevine (Ball *et al.* 2020; Marks *et al.* 2022). Where traditional management practices (herbicide and tillage) have impacted negatively on soil organic carbon (SOC) concentrations, the adoption of cover crops (mid-row and under-vine) can be seen as a means of increasing and retaining SOC through the implementation of natural feedback loops (Ball *et al.* 2020; Fleishman *et al.* 2021). Moreover, the pressure to adopt alternative vineyard floor management practices has increased – certainly in Europe – with the adoption of policy banning the use of glyphosate herbicides (Jacquet *et al.* 2021).

Carbon is a ubiquitous element in the biotic world. Plants (and other photosynthetically-capable organisms) are able to capture carbon dioxide (CO_2) molecules and convert them into carbohydrates (Lal 2020). Plants capture CO_2 from the atmosphere, and H_2O and nutrients from the pedosphere (soil). It is predominantly this active conduit that has enabled some 1500 Pg of C to become translocated and stored in the earth's soils (Lal *et al.* 1997). The active increase and retention of SOC in agroecosystems, such as vineyards, has numerous environmental and economic benefits. Soil organic carbon sequestration can help to reduce atmospheric carbon content, thus aiding in the mitigation of climate change (Ball *et al.* 2020; Marks *et al.* 2022). In the shorter term – and not unlike cover crop use – SOC sequestration has been shown to improve soil stability, water availability, nutrient-retention and soil biological processes, among much else (Novara *et al.* 2019; Ruiz-Colmenero *et al.* 2013). Combining ideas around vineyard floor management and the importance of SOC in vineyards, we can begin discussing the role of cover crops as tools for the increase and retention of vineyard SOC. Several notable studies have sought to quantify the effects of SOC concentrations, stocks and turnover, both in the mid-row (Peregrina *et al.* 2010; Steenwerth & Belina 2008) and under-vine (Ball *et al.* 2020; Fleishman *et al.* 2021; Marks *et al.* 2022). Targeted and site-specific approaches are essential, with the adoption of vineyard floor strategies reliant upon both biotic conditions (grapevine) and abiotic conditions (climate, rainfall, irrigation, etc.) (Marks *et al.* 2022). In the latter, we might also include vineyard topography and whether cover crop use may provide a mitigation strategy against erosion (Ruiz-Colmenero *et al.* 2013). With growing interest from viticulturists and consumers on increasing vineyard SOC, there comes a need for greater understanding of SOC in vineyards.

The primary aim of this review will be to synthesise the current scientific knowledge surrounding the influence of vineyard floor management practices – namely cover crops – on SOC concentrations and stocks in vineyards. Organically-derived carbon molecules come in many shapes and sizes and their residence time within vineyard soils may differ by orders of magnitude, depending on their above- and below-ground source (Hoffland *et al.* 2020). Understanding the residence time and decomposition rate of SOC fractions (Baldock *et al.* 2018) is important both in terms of nutrient-cycling and carbon turnover but also to inform vineyard managers on which practice will suit a desired outcome (Marks *et al.* 2022). Therefore, in addition to SOC stocks and concentrations, this review will also compare and contrast research into specific SOC composition – focusing on physical fractions (coarse and fine) and chemical fractions (labile and recalcitrant) (Ball *et al.* 2020; Peregrina *et al.* 2010). Within this framework, we will also explore carbon turnover – specifically, how microbial activity responds to different management practices, which can provide a proxy for carbon turnover (Marks *et al.* 2022). It is hoped that by the end of this review, the reader will have both a broader and more specific understanding of the role of vineyard floor management and its influence on various SOC dynamics, both in the mid-row and under-vine. We also seek to inform future efforts, and so conclude with the proposal of potential future research to better understand SOC dynamics in vineyards.

Before going further, it should be noted that this review pertains directly to SOC as an instrument to influence ecosystem services in vineyard soils. Discussions around C sequestration in relation to carbon trading and offsetting are certainly noteworthy; however, are subject to change with governmental regulation and are not within the scope of this review. Therefore, all mention of C sequestration and SOC dynamics shall be discussed only in relation to its biological and ecological influences within vineyard systems.

2.2 Vineyard floor management and soil organic carbon

Converting land-use from a natural system to an agroecosystem requires the removal of plant biomass and the tilling of soil to prepare for the cash crop (Steenwerth & Belina 2008). All of these actions have the side-effect of depleting soil organic carbon (SOC) and disrupting soil structure and aggregate stability (Ruiz-Colmenero *et al.* 2013). The physicochemical and environmental benefits of SOC increase and retention in all soils – in this case, vineyard soils – is well-understood. Increasing and retaining SOC in vineyard systems can be achieved passively, by minimal intervention (reduced soil disturbance) (Gómez *et al.* 2011). Actively increasing (and retaining) SOC in vineyards can also be achieved through the implementation of several vineyard floor management practices (Marks *et al.* 2022). This section (and most this review) will focus on research conducted on the use of cover crops as tools to both increase and retain SOC in vineyard systems.

2.2.1 *Life under the vines*

The sowing of cover crops directly under-vine; however, has been less swiftly and wholly adopted by viticulturists and vineyard managers as compared to mid-row use. Perhaps the greatest concern among viticulturists operating in warm, dry (*Mediterranean-type*) climates is that pertaining to plant-available water and thus the potential for competition between grapevine and cover crop at the root zone (Costello 2010; Delpuech & Metay 2018). Fear of competitive inhibition, therefore, has long seen the under-vine section managed with herbicides in order to maintain bare earth and reduce competition (Ball *et al.* 2020; Karl *et al.* 2016). A drought of research on under-vine management has broken in recent years, with several important studies seeking to demystify under-vine soil-grapevine interactions (Ball *et al.* 2020; Fleishman *et al.* 2021). The adoption of particular under-vine management practices (cover crops, mulch, compost, etc.) appears concomitant with both desired outcomes (end product) and the distinct nature of vineyard terroir (biotic and abiotic vineyard variables) (Bramley 2022). One such outcome that has seen a pronounced rise in both scientific and policy-based interest is that pertaining to the *increase* and *retention* of soil organic carbon (SOC) in vineyard agroecosystems (Ball *et al.* 2020; Peregrina *et al.* 2014; Ruiz-Colmenero *et al.* 2013).

2.2.2 *To increase or to retain? A targeted approach with cover crops*

To cover crop or not to cover crop; that is the question… or perhaps the more pertinent question should ask: *if* one employs cover crops, is it to *increase* or *retain* SOC in the vineyard floor? Under this line of inquiry, the desired outcome determines the approach. The latter approach – SOC retention – appears most often employed in hot, dry, sloping vineyards (Marques *et al.* 2010; Ruiz-Colmenero *et al.* 2013). Retention, therefore, refers to the mitigation of SOC loss by erosion via the use of cover crop root systems that act to stabilise soil, while above-ground biomass reduces the dispersive force of rainfall (Marques *et al.* 2010).

Studies conducted on sloping vineyards in the Mediterranean (notably, western Sicily and central Spain) highlight the positive use of cover crops to prevent SOC loss (Novara *et al.* 2019; Ruiz-Colmenero *et al.* 2013). The Sicilian study utilised a sloping vineyard trial site that was divided in half, with one treatment composed of whole-floor cover crops and the other conventional tillage (bare earth) (Novara *et al.* 2019). Sampling the upper, middle and lower slopes, the authors found that after five years the total SOC concentration was 8.74 ± 0.20 g kg^{-1} under conventional tillage and 9.52 ± 0.34 g kg^{-1} under cover crops on the sloping section (Novara *et al.* 2019). Moreover, in olive orchards and vineyards, Gómez *et al.* (2011) reported a ratio of SOC retention ranging between 1.7 and 36.9 between conventional tillage and cover crops respectively. Converse to retention, they further reported SOC loss under conventional tillage of 27 kg ha $^{-1}$ and 392 kg ha $^{-1}$ on gradients of 4% and 11% respectively (Gómez *et al.* 2011).

A second study on a sloping vineyard in central Spain aimed to determine whether two types of cover crop had a positive impact on SOC retention (erosion prevention) when tested against conventional tillage (bare earth) (Ruiz-Colmenero *et al.* 2013). As with the Sicilian study, Ruiz-Colmenero *et al.* (2013) found significantly higher SOC stocks in both mid-row cover crop treatments than the conventional tillage (11.7 and 11.5 t ha^{-1} respectively, versus only 8.0 t ha^{-1} under conventional tillage). This study was multifaceted and, in addition to measuring SOC retention, the authors also measured sediment and SOC loss (t ha^{-1}). Employing a sediment trap beneath each treatment, the study allowed 48 rainfall events to pass. Over six times as much sediment was found in traps beneath conventionally tilled treatments versus both cover crops, demonstrating the ability of cover crops to stabilise soil and protect against rainfall impact (Ruiz-Colmenero *et al.* 2013). Soil organic carbon was 1.4 times higher in cover crop sediment traps even though erosivity was far less. This is very likely due to higher rates of SOC sequestration under cover crop treatments, owing to increased plant biomass (Ruiz-Colmenero *et al.* 2013). This last point is of particular importance to this review – whether the aim is to *increase* or *retain* SOC, really, each target is one side of the same coin. Utilising cover crops for SOC retention will, by their mere presence, also increase the amount of SOC through primary production and biomass turnover.

2.2.3 *Additional amendments: mulch, compost and biochar*

Cover crops account for only one aspect of vineyard floor cover, with mulches, composts and, more recently, biochar often employed to stabilise soil, reduce temperature and improve water infiltration (Lazcano *et al.* 2020; Prosdocimi *et al.* 2016). The practices have also been shown to both increase and retain SOC content compared to conventional bare earth (Prosdocimi *et al.*

2016). The physicochemical attributes of straw mulch (lignin content and high C:N ratio) prevent rapid degradation (Fanin *et al.* 2021). Owing to this, data on the use of straw mulch is mostly concerned with SOC retention through soil stabilisation, reduced rainfall impact and improved infiltration leading to reduced erosion (Jordán *et al.* 2010; Prosdocimi *et al.* 2016). Trials involving the application of compost (animal or plant-based litters) appear mostly concerned with increasing SOC, owing to the higher organic carbon content and more labile pools entering the soil (Lazcano *et al.* 2020). Several studies have shown a positive increase in SOC stocks through the use of cover crop composts versus bare earth treatments, with a linear relationship established between the amount of compost and concentration of SOC (Mondini *et al.* 2018; Morlat & Chaussod 2008). A study in the Adige Valley in Italy tested the effects of three fertilizer amendments, including a mineral fertilizer, bovine manure and green manure (Morelli *et al.* 2022). Although the study was unable to discern differences in mid-row SOC concentrations, the authors did find differences in SOC composition, with green manure (cover crop application) increasing recalcitrant (stable) SOC over a six-year period (Morelli *et al.* 2022).

A more recent alternative vineyard floor management practice pertains to the use of biochar – the carbonaceous residue resulting from various methods of pyrolysis (Sohi *et al.* 2010). Biochar – as an inert form of OC – is considered largely non-bioavailable and thus its addition to soil means that its residence time is greater than more labile forms of OC (Rombolà *et al.* 2015; Sohi *et al.* 2010). Although biochar is largely inert, it does contain large quantities of DOC, which can become solubilised and leached, causing a flux in microbial availability (Liu *et al.* 2019) Biochar physicochemical matrices are able to increase SOC retention through increased binding sites, thus reducing bioavailability of C molecules (Rombolà *et al.* 2015). This last point is suggestive that biochar may aid in both carbon sequestration and environmental remediation (Gelardi & Parikh 2021). The authors propose that trial-based inquiries across multiple soil types and climates is necessary to increase the depth of knowledge on the influences of biochar in both natural and agricultural systems (Gelardi & Parikh 2021).

2.3 Taking stock

Specifically, SOC stocks are a measure of the concentration of organic carbon contained in a known volume of oven-dried soil (Al-Shammary *et al.* 2018). Extrapolating from sample cores (SOC concentration and bulk density), SOC stocks are typically presented in tonnes of C per hectare of soil (adjusting for sample depth) (Al-Shammary *et al.* 2018). Methodological approaches to quantifying SOC stocks will be considered in more detail in section 3.2; however, it is worth mentioning an important principal of pre-sample preparation. This regards both the quantification of SOC in terms of a treatment-based approach (i.e. X-management practice vs Y-management practice) or as a sequestration-based approach (i.e. X-management practice vs Y-management at time A, B, C…). In both cases, it is important to establish a so-called *baseline* of SOC for each treatment (Nayak *et al.* 2019). Establishing a baseline is necessary to form a boundary point by which changes in SOC stocks over time may be referenced to a starting point (Olson 2013). This is perhaps more important if considering a longitudinal trial, where sequestration is being quantified – rather than a purely treatment-based trial – or until C-saturation and a steady-state has been reached (Cavagnaro *et al.* 2016; Nayak *et al.* 2019).

Quantifying carbon stocks in natural or agroecosystems has benefits toward long-term carbon sequestration and SOC modelling (Holmes *et al.* 2012; Powlson *et al.* 2012). Several studies have sought to quantify carbon stocks in vineyard ecosystems, with significant results for SOC accumulation (Galati *et al.* 2016; Novara *et al.* 2019). Morandé *et al.* (2017) quantified C stocks in a Californian vineyard through measurements of trunks, roots, leaves and fruit C content (Morandé *et al.* 2017). They quantified total vine C stocks as 12.3 Mg C ha^{-1}, with 8.9 Mg C ha^{-1} attributed to perennial grapevine biomass (Morandé *et al.* 2017). Most vineyard C stocks fall into the category of vine-derived biomass, however this technique can be paired with SOC measurements for a holistic overview. Moreover, these results demonstrate how well vineyards can act as both C sinks but also as reliable systems for C quantification (Brunori *et al.* 2016; Holmes *et al.* 2015; Morandé *et al.* 2017).

Several studies have quantified SOC stocks in agroecosystems, yet few in vineyards. Holmes *et al*. quantified total organic carbon (TOC) in kiwifruit orchards and developed a robust model for quantifying TOC in vine-based agroecosystems (Holmes *et al.* 2015). Establishing 4-10 soil cores at varying depths, they were able to quantify TOC with an 80% accuracy (Holmes *et al.* 2015). Brunori *et al.* (2016) quantified TOC stocks from a range of environments (natural, commercial and peri-urban), with vineyard agroecosystems constituting a high percentage of commercial sites. The authors quantified TOC stocks at 73.35 t C ha^{-1} in organic (cover crop) vineyards and 44.16 t C ha^{-1} in conventionally-tilled vineyards (Brunori *et al.* 2016). These figures were equivalent to TOC stocks measured in certain forest ecosystems, showing the potential of vineyards to both act as C sinks and also their reliability as perennial (woody biomass), relatively undisturbed systems.

The inclusion of cover crops in agroecosystems can increase SOC stocks to the effect of increasing ecosystem services, while mitigating greenhouse gas (GHG) emissions (Olson *et al.* 2014; Poeplau & Don 2015). Through a meta-analysis, Poeplau & Don (2015) estimated potential global C storage by cover crops to be in the order of 0.12 ± 0.03 Pg C yr^{-1} in agricultural systems (Poeplau & Don 2015). Their estimate is admittedly rough, in part, due to a paucity of data – a clear knowledge gap in cover crop-related C stocking. Olson *et al.* (2014) conducted SOC measurements on degraded soil (plow tilled) and control (no till) using both cover crops and no cover crops (treatments). Their sampling was conducted over a 12-year period and reported significantly higher accumulation of SOC stocks in all cover crop treatments (Olson *et al.* 2014). A key message from their study pertained to careful sampling over a long period of time as the best method to understand how SOC is accumulated in remediated soils.

2.3.1 *Taking stock under-vine*

The majority of studies have focused on quantifying SOC concentrations and stocks in the vineyard mid-row, with only a limited number measuring SOC concentrations under-vine (Ball *et al.* 2020; Fleishman *et al.* 2021). A recent exception to this regards a recent publication by Marks *et al.* (2022), whose research quantified differences in SOC stocks under-vine across two vineyards in South Australia. The study compared two traditional under-vine practices (herbicide and straw mulch) and two cover crop combinations (*Medicago*: ryegrass mix and fescue: clover mix). Sampling to a depth of 30 cm, the authors determined that SOC stocks were up to 23% higher under treatments of cover crop versus the herbicide-managed control over a five-year growth period (2014 – 2019) (Marks *et al.* 2022). A previous under-vine trial across four South Australia vineyards showed strong evidence to suggest that cover crops sown under-vine positively influence both SOC and mineral N concentrations (Ball *et al.* 2020). The study tested the effects of four treatments, including one grass, one legume, a grass with legume mix and a herbicide-managed bare earth control (Ball *et al.* 2020). Focusing on the top 0-10 cm, the authors reported a 14% increase in SOC (mg kg^{-1}) under treatments of grass versus the bare earth control (Ball *et al.* 2020). Moreover, they were able to correlate increased root biomass with increase SOC concentrations under-vine, with grass biomass 22% greater than legume biomass – a characteristic well-recognised in previous literature (Ball *et al.* 2020; Puget & Drinkwater 2001). Interestingly, grass root biomass and therefore SOC concentrations were greater in treatments of grass with legume owing to the mobilisation of N by leguminous cover crops (Ball *et al.* 2020). Not unsurprisingly, root biomass appears to be a greater overall contributor to SOC retention and increase than shoot biomass, accounting for approximately 2.4 times the amount of plant-derived organic C (Ball *et al.* 2020; Rasse *et al.* 2005).

A second under-vine cover crop trial in Pennsylvania, USA, tested the impact of a grass cover crop versus a herbicide-managed bare earth control (Fleishman *et al.* 2021). Similar to the previous study, theirs focused on concentrations of SOC rather than stocks; however, with the key difference that sampling depth was stratified to 1 m, with the first 0-40 cm stratified at 10 cm intervals (Fleishman *et al.* 2021). The key finding from this research was that a grass cover crop appreciably increases SOC concentrations in shallow soil but less so at depth (i.e. beyond the root zone) – a finding consistent with other, mid-row studies (Celette *et al.* 2005; Fleishman *et al.* 2021). Both studies confirmed the positive influence of grass cover crops to increase SOC under-vine, with the key relatable variable being increased root mass correlating with higher SOC concentrations (Ball *et al.* 2020; Fleishman *et al.* 2021). Both, however, also described an overall decrease in mineralisable N (NO_3^- and NH_4^+) within the root zone, owing to increased microbial activity and therefore a net immobilisation of N (Ball *et al.* 2020; Fleishman *et al.* 2021). Although this finding was stated in both studies, only Ball *et al.* (2020) were able to further assert that a leguminous cover crop (or cover crop mix) may be able to counteract mineralisable N-species depletion through legume-rhizobium root nodulation, leading to N-fixation. This finding highlights the potential importance of sowing combinations of cover crop – each with contrasting functions. In the aforementioned study, the leguminous cover crop provides its grass-species partner the requisite N to increase root mass (while not diminishing grapevine N), thus leading to increased SOC concentrations and soil stability (Ball *et al.* 2020).

2.3.2 *Plumbing the depths*

The decision on sampling depth can vary widely and is primarily dependent on several factors, including experimental design and the ecosystem in question. The prevailing understanding is that the vast majority of SOC is concentrated in the upper soil profile – between 0-30 cm but predominantly in the top 10 cm – around the bulk rhizosphere and therefore the region where the majority of microbial activity is concentrated (Fleishman *et al.* 2021; Wiesmeier *et al.* 2019). Owing to this, the majority of studies seeking to quantify SOC concentrations and SOC stocks in agroecosystems are concerned with the top 0-10 cm and 10-30 cm, where the majority of crop (and cover crop) roots are found and therefore where most N-cycling and C-turnover persist (Ball *et al.* 2020; Payen *et al.* 2020; Steenwerth & Belina 2008). Studies focused on natural systems – predominantly those dominated by deep-rooted perennials – are more often concerned with C stores over a deeper range (up to 1 m) (Cusack & Turner 2021; Hou *et al.* 2019; Rasmussen *et al.* 2018). This appears to be both due to the presence of deeper root systems contributing to SOC dynamics at depth *and* to the historical legacy of such ecosystems, allowing sufficient time to increase C activity at greater depth (Hou *et al.* 2019; Stockmann *et al.* 2013). Stratifying SOC measurements by depth – and indeed, sampling deeper – can provide a more nuanced understanding of C composition within an ecosystem (Fontaine *et al.* 2007; Jobbágy & Jackson

2000). Carbon stored at depth, beyond the bulk root zone (and thus microbial community), is typically thought to be more stable and resistant to decomposition – perhaps several orders of magnitude so, according to Fontaine *et al.* (2007).

Concerning agroecosystems – namely, vineyards – sample depth is perhaps as a much a decision constrained by logistics as by hypotheses concerning deep C stores and a desire to quantify them. That is not to suggest that quantifying deep-lying SOC in vineyards (and other agroecosystems) is not beneficial, nor worthwhile. However, obtaining sample cores to a depth of 1 m can present difficulties of its own and the information gained is perhaps less beneficial than in those deep-rooted, natural ecosystems, such as forests. Such logistical constraints are perhaps most pronounced when sampling the under-vine area of vineyards as opposed to the mid-row), where manoeuvrability is more cumbersome. Thus, most under-vine SOC studies have focused on a depth of between 0-30 cm (but mostly within the top 10 cm), with the notable exception of research conducted by Fleishman *et al.* (2021), who stratified sampling to a depth of 40 cm in order to specifically compare the effects of cover crop management on shallow vs deep under-vine SOC. Their results showed that although cover crops substantially increased shallow SOC concentrations, there was little impact on deeper (20-40 cm) SOC stores (Fleishman *et al.* 2021). Although only one study, such results highlight a practical trade-off between the viability of the data collected vs the cost and effort of sampling at greater depth.

2.4 Forms and pools…not all carbon is built equal

Quantifying soil organic carbon (SOC) concentrations and stocks provides valuable information on *how much* organic-derived carbon is present within a vineyard system and over a certain depth. The next logical question might then ask: *what kind* of carbon is present? Combining the two measurements would then inexorably lead to: *how much* of *what kind* of carbon exists in a vineyard system and over a certain depth? With each question answered, the SOC picture becomes more complete and informative. Fractions of SOC (physical and chemical) differ in response to the above- and below-ground litter inputs (Guo *et al.* 2021). Plant litter that enters the soil may do so in either a particulate or dissolved form. In the case of vineyard ecosystems, particulate litter may be grapevine-derived, such as leaves, grapes, stems and sloughed bark; or cover crop-derived (annual senescence) (Hoffland *et al.* 2020). Dissolved litter forms may enter the soil by root exudates, grape-derived sugars or rhizodeposits (see Fig. 1) (Hoffland *et al.* 2020). Both the diversity and abundance of ecosystem litter type affects SOC functional diversity and, therefore, foliar *and* soil microbial communities (Chapman & Newman 2010). Litter types – specifically, with regards to those characteristics that dictate the *rate* and *totality* of decomposition (and therefore SOC composition) – can be viewed on a spectrum; from *labile* (more easily metabolised) to *recalcitrant* (less easily decomposed) (Suseela *et al.* 2013). Furthermore, physical fractionation of soil can provide a robust understanding around the longevity and potential turnover rate of SOC in vineyard soils (Ball *et al.* 2020). Coarse soil fractions are more susceptible to change and so provide a relative gauge for SOC turnover, while fine fractions have likely undergone microbial decomposition to reach a stable equilibrium (Ojeda *et al.* 2018).

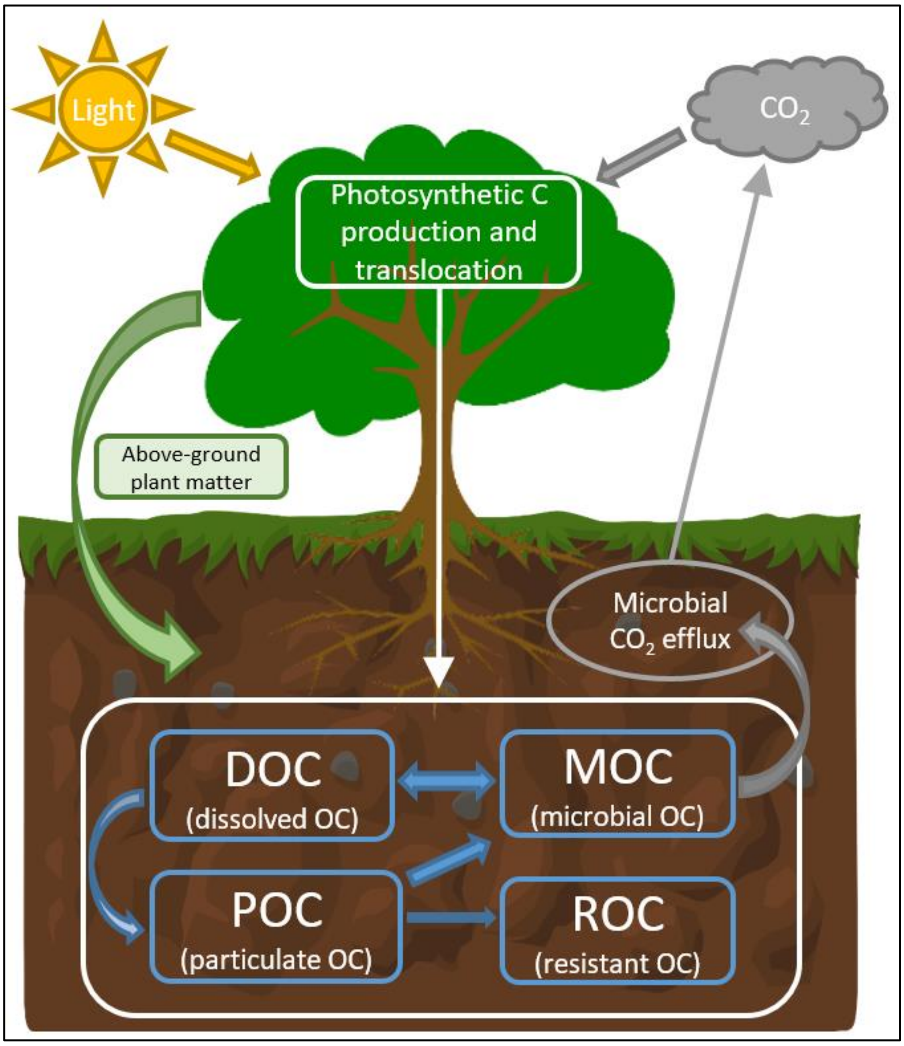


Figure 1. Conceptual diagram of SOC pathway, from primary production to root-translocation. Soil organic carbon pools are represented, as well as SOC flow between pools and microbial efflux back into the atmosphere.

2.4.1 *Food for thought: labile carbon and the microbial perspective*

Organic-derived carbon can be separated into several pools based on size and chemical signature. Labile carbon, even within itself, is a term wedded to a spectrum (Cheng *et al.* 2007). To what extent we can separate various labile pools is a function of those methodologies available. Perhaps the most labile forms of organic carbon (OC) – and indeed the simplest to measure – are dissolved organic carbon (DOC) and particulate organic carbon (POC) (García-Díaz *et al.* 2018; Peregrina *et al.* 2010). These pools represent the most microbially-favourable, owing to their size, chemical composition and therefore ability to become readily metabolised (Peregrina *et al.* 2010). The relatively rapid turnover of DOC and POC can make them useful indicators for microbial activity and therefore short term change – as may be the case when comparing vineyard floor management practices (i.e. cover crop use vs tillage) (Novara *et al.* 2014; Peregrina *et al.* 2010; Steenwerth & Belina 2008).

Several studies have sought to quantify labile OC pools in response to vineyard floor management practices, with a large emphasis placed on cover crop use versus "traditional" techniques. A mid-row study based in La Rioja region of central Spain quantified concentrations of both DOC and POC from underneath three treatments: two cover crops and one conventional tillage (Peregrina *et al.* 2010). Results from the study showed significantly ($p < 0.05$) increased concentrations of both DOC and POC concentrations under treatments of cover crop versus conventional tillage (3-fold greater) in the top layer (0-2.5 cm) (Peregrina *et al.* 2010). Concentrations of POC remained significantly higher in cover crop treatments from 2.5-15 cm; however, those differences were markedly finer than at 0-2.5 cm (Peregrina *et al.* 2010). Similarly, differences in DOC were considerably higher at 0-2.5 cm depth and then only significantly so at a depth of 5-15 cm (Peregrina *et al.* 2010). The authors' results accord with similar findings from several other studies, where surface residue inputs account for the highest concentrations of labile OC (Álvaro-Fuentes *et al.* 2008). Significant differences in OC at depths between 5-15 cm are likely root-derived (exudates and rhizodeposits); however, these account for a much smaller proportion of the overall labile pool (Franzluebbers & Stuedemann 2002; Peregrina *et al.* 2010).

Measuring heterotrophic respiration as a function of metabolic activity has been used as a proxy for the presence of labile OC in a soil ecosystem (Marks *et al.* 2022; Peregrina *et al.* 2010; Steenwerth & Belina 2008). A study across two South Australian vineyards compared cumulative respiration and DOC stocks from soils sampled from beneath two traditional under-vine practices (herbicide and straw mulch) and two cover crop combinations (medic: ryegrass mix and fescue: clover mix) combinations (Marks *et al.* 2022). Although cumulative respiration was found to be 2–2.5-fold higher under cover crop treatments, DOC stocks were only significantly different at one vineyard site, where increased rates of irrigation may have increased solubilised OC (DOC) (Marks *et al.* 2022). A mid-row study in a Californian vineyard tested the effects of two cover crops versus a conventionally-managed treatment (Steenwerth & Belina 2008). Again, DOC was selected as the labile OC pool, while microbial biomass and CO_2 efflux were also measured as proxies for the presence of labile OC (Steenwerth & Belina 2008). In this case, both microbial activity and DOC fluctuated seasonally, responding to both labile OC and soil moisture content (Fierer & Schimel 2002; Steenwerth & Belina 2008). Microbial biomass and CO_2 efflux were reported to be 2–4 and 2–6-fold greater under both cover crops respectively, while DOC was 1.5–3-fold times higher under both cover crop treatments versus cultivation (Steenwerth & Belina 2008). Positive correlations between labile OC and microbial activity (biomass and CO_2 efflux) are echoed in other vineyard floor studies, with each suggestive that labile OC pools are most influential in stimulating microbial activity but also fluctuate with soil moisture content (seasonally or irrigation regime) (Marks *et al.* 2022; Novara *et al.* 2014; Peregrina *et al.* 2010; Steenwerth & Belina 2008). Within both the Sicilian and Californian studies, labile OC concentrations were observed to be highest in upper soil layers (0-2.5 cm and 0-15 cm respectively) – a finding that accords well with previous understandings of labile OC dynamics in vineyard systems (Ball *et al.* 2020; Peregrina *et al.* 2010; Steenwerth & Belina 2008).

2.4.2 *Never say die: recalcitrant carbon, biochar and grapevine woody biomass*

Far fewer studies have sought to quantify the presence of recalcitrant – *or resistant* – OC with respect to vineyard floor management. One reason for this is that quantifying SOC on a spectrum of recalcitrance can be exceedingly difficult, with several metrics often employed and the results often challenging to interpret (Cheng *et al.* 2007). Physical and chemical fractionation methods are commonly used; however according to Cheng *et al.* (2007) these are not altogether reliable, with certain recalcitrant chemical signatures appearing in both fine and coarse SOC fractions. Indeed, another and perhaps more practical reason is that labile OC is both a more informative indicator of change in vineyard floor management practice *and* that recalcitrant OC requires a significantly longer amount of time to form and accumulate (Ball *et al.* 2020; Paul *et al.* 2001).

This second point presents logistical issues regarding relatively short-term vineyard floor management trials – both in terms of observable treatment differences and the depth to which sampling occurs (Dalal *et al.* 2021; Marks *et al.* 2022). This is especially relevant in trials involving cover crop treatments, where the majority of SOC deposited is both labile (low lignin content) and at a shallow depth (Dalal *et al.* 2021). Therefore, investigations into recalcitrant SOC in vineyards is perhaps most aptly conducted where vineyard floor management involves the use of highly lignified amendments, such as grapevine canes or the use of inert C, such as with biochar (Morlat & Chaussod 2008; Rombolà *et al.* 2015).

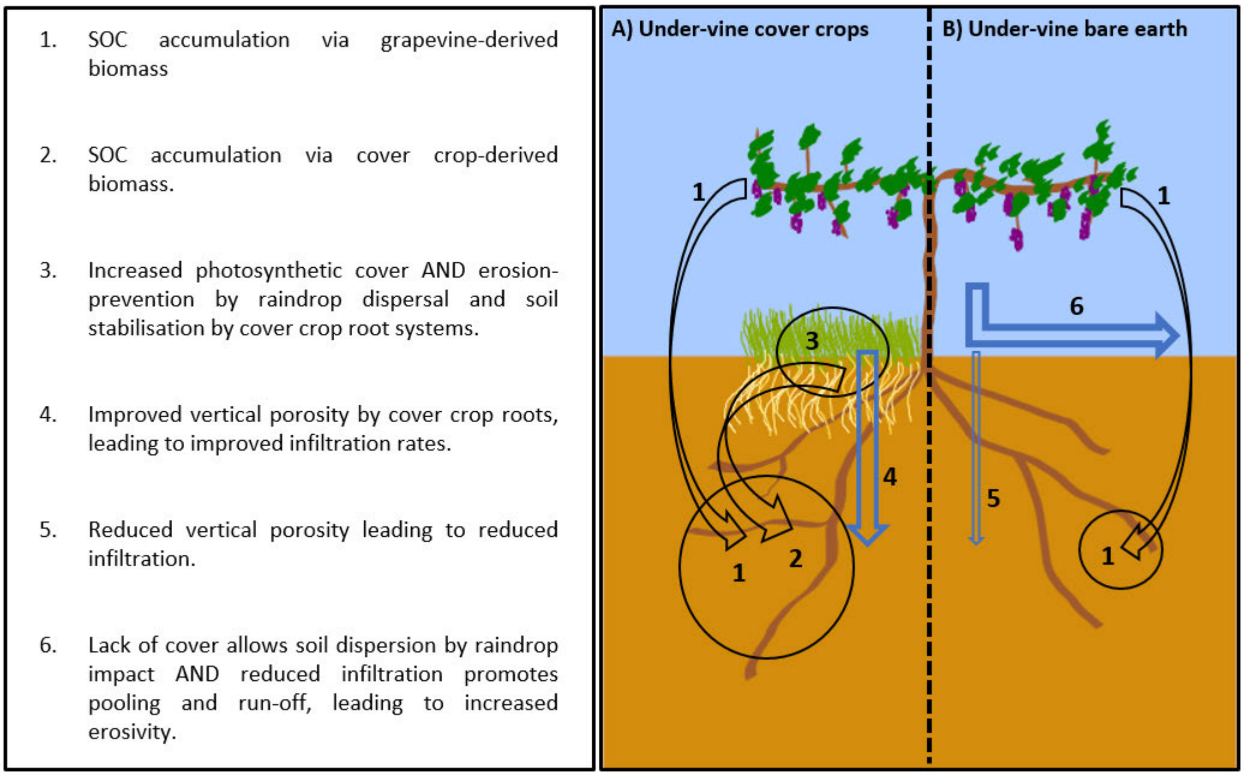
The use of grapevine biomass (predominantly canes) as an organic amendment to increase SOC has merit; however, few studies have sought to explore is efficacy (Morlat & Chaussod 2008). An immediate benefit of employing grapevine vegetative biomass as an organic amendment is that it requires no transport effort and therefore relatively little carbon cost. One notable study undertaken in the Loire Valley of central France quantified SOC sequestration between 1976 and 2004, utilising several organic amendments, including dried and crushed grapevine canes (2 t/ha/yr), spent cow manure and mushroom compost (Morlat & Chaussod 2008). Within the first 11-years of sampling, only the cow manure and mushroom compost were significantly higher in total organic carbon (TOC) versus both the crushed canes and unamended control (Morlat & Chaussod 2008). However, after 23 and 28 years respectively, soils amended with crushed canes were statistically similar in TOC content to both the manure and compost, with all three higher than the unamended control (Morlat & Chaussod 2008). The decadal delay in detectable TOC under treatments of crushed canes versus those of manure and compost is likely a result of the physicochemical nature of the cane biomass – that being higher lignin content and thus requiring far longer to breakdown and show significant effects on SOC levels (Morlat & Chaussod 2008). Grapevine biomass can also be used to generate biochar – an amendment with physicochemical properties that can both *increase* and *retain* SOC in vineyards (Rombolà *et al.* 2015; Sohi *et al.* 2010). As mentioned in section *3.3*, biochar represents a relatively inert, stable form of OC, owing to its pyrolytic processing and therefore non-bioavailability (Sohi *et al.* 2010). Biochar not only increases SOC (mostly in the recalcitrant form) but also assists in trapping OC and therefore plays the dual role of increaser and retainer, owing to its complex matrix structure and increased surface area (Rombolà *et al.* 2015). Simultaneously, the role of biochar in protecting OC molecules from decomposition may be an unwanted side-effect of its use as a vineyard amendment (Quilliam *et al.* 2013; Rombolà *et al.* 2015). As Rombolà *et al.* (2015) note, the complex matrix of biochar – so adept at decreasing the bioavailability of OC – may also serve a negative purpose in preventing certain deleterious polyaromatic hydrocarbons from becoming suitably degraded in soils. As an addendum, it should be noted that in order to formally *sequester* OC using C-rich amendments, those amendments must derive from the land upon which the sequestration occurs (Badgery *et al.* 2020).

2.4.3 *A confluence of rivers: quantifying the co-benefits of SOC*

Please permit, just for a moment, a brief stray into the esoteric… Soil organic carbon, in this metaphoric chapter title, is the confluence of rivers, the grand yet all-too-often ill-meeting of environmental sustainability and economic prosperity. Too often where these two meet, they do so antagonistically, but not this time. Previously we discussed the targeted approach – whether to *increase* or to *retain* SOC. In this, we concluded that these approaches are but two sides of the same coin – the implementation of one will initiate the action of the other. In the same breath we can then begin to discuss the so-called *co-benefits* of SOC, with a particular emphasis on the influence of cover crops and SOC on soil stability and water dynamics.

2.4.4 *Soil structure and aggregate stability*

The dominant co-benefit of increasing/retaining SOC in a vineyard using cover crops relates to the multi-layered effect on soil structure and aggregate stability (see Fig. 2) (Peregrina *et al.* 2010; Ruiz-Colmenero *et al.* 2013). At the molecular level, SOC provides increased negative binding sites which allow polyvalent cations, such as Mg^{2+} and Ca^{2+} to form cation bridges, forming micro-aggregates (Brady 2008; Bronick & Lal 2005). This in turn promotes the formation of larger aggregates which become stabilised further with the aid of larger OC fractions such as mucilage, roots and fungal hyphae (Brady 2008; Bronick & Lal 2005). A vineyard cover crop trial in La Rioja region of Spain found that soils planted with cover crops had improved water aggregate stability versus conventional tillage to a depth of 0-2.5 cm (Peregrina *et al.* 2010).



1. SOC accumulation via grapevine-derived biomass
2. SOC accumulation via cover crop-derived biomass.
3. Increased photosynthetic cover AND erosion-prevention by raindrop dispersal and soil stabilisation by cover crop root systems.
4. Improved vertical porosity by cover crop roots, leading to improved infiltration rates.
5. Reduced vertical porosity leading to reduced infiltration.
6. Lack of cover allows soil dispersion by raindrop impact AND reduced infiltration promotes pooling and run-off, leading to increased erosivity.

Figure 2. Conceptual diagram representing under-vine management strategies: cover crop use (left) and herbicide-management (right). Numbering corresponds to legend (left) and represents the multifarious co-benefits of cover crops to increase and retain SOC in vineyards, as well as performing key ecosystem services.

The same study found that both DOC and POC were also increased under the same treatments and to the same depth (Peregrina *et al.* 2010). Cover crops not only promote an increase in vineyard SOC – and therefore soil stability – but also act as soil stabilisers in themselves (Novara *et al.* 2019; Ruiz-Colmenero *et al.* 2013). Vineyard floor studies concerning the mitigation of erosion – typically sloping vineyards in Mediterranean-type climates – have tested the role of cover crops in preventing topsoil loss (see Fig. 2) (Novara *et al.* 2019; Ruiz-Colmenero *et al.* 2013). A Sicilian study on a sloping vineyard site found that treatments of cover crops (*Vicia* *faba*) retained 6% OC on the flat section and 9% OC on the sloping section versus bare earth control (Novara *et al.* 2019). As previously mentioned in section 2.2, a study in La Rioja showed that cover crop canopy and roots maintained a dual action, preventing erosion and sediment six-times more effectively than conventional tillage (Ruiz-Colmenero *et al.* 2013). A similar study on a sloping vineyard in NE Spain demonstrated the effectiveness of cover crops to prevent sediment transport (López-Vicente *et al.* 2020).

Previously cited studies predominantly focused research on vineyards in hot, dry climates. However, several studies have reported the positive effects of ground cover (wild or seeded) on vineyard soil stability in typically wet climates (Vanden Heuvel & Centinari 2021). These studies have understood the use of groundcover (under-vine and mid-row) in much the same way – as a tool to mitigate raindrop soil dispersion, runoff and erosion, while also reducing grapevine vigour (Vanden Heuvel & Centinari 2021). Two notable studies focused on the Finger Lakes region of the Atlantic northeast – a region prone to high rainfall events – with findings suggestive that permanent groundcover greatly improves soil aggregate stability (Chou & Heuvel 2019; Karl *et al.* 2016). In the earlier of the two studies, Karl *et al.* (2016) tested various soil structural characteristics between treatments of white clover, native vegetation and traditional, bare-earth practices. Their results found that in the fourth year of the trial traditionally cultivated soils had increased bulk density, lower porosity and WHC than other treatments, while both groundcover treatments were found to have 46% greater aggregate stability than cultivated soil (Karl *et al.* 2016). In the later study, Chou and Heuvel (2019) found that treatments of natural vegetation increased soil structural stability by 82% versus soil treatments managed with glyphosate.

2.4.5 *Water holding capacity and infiltration*

In a similar breath to discussions regarding soil structure and stability, we can look to assessing the influence of SOC and cover crops on certain soil water dynamics; namely, plant available water capacity (PAWC) and infiltration (see Fig. 2). Water-holding capacity in soils is a function of several physicochemical properties, namely soil texture (ratio of sand, silt and clay) and soil porosity (size and distribution) (Acín-Carrera *et al.* 2013; Brady 2008). Additionally, SOC (or

SOM) is understood to be a key contributing factor to increasing PAWC in soils, either directly or by improving soil structure and aggregate stability (Lal 2020). Water use efficiency is an ongoing concern in hot, dry climates, where water may be limiting (Marks *et al.* 2022; Ruiz-Colmenero *et al.* 2013). As well as increasing SOC content and therefore contributing to improvements in PAWC, cover crop use can also enhance infiltration rates in vineyards (Ruiz-Colmenero *et al.* 2013). Improving infiltration promotes water to travel down to the root zone where it is needed, rather than pooling on the surface where it is either evaporated or increases the chance of lateral run-off, contributing to erosion and topsoil loss (Chalise *et al.* 2018; Ruiz-Colmenero *et al.* 2013). Vineyard floor management in the Mediterranean Basin traditionally relied heavily on tillage regimes designed to break soil crusts, reduce weed competition and manually improve infiltration (Ruiz-Colmenero *et al.* 2013). Ruiz-Colmenero *et al.* (2013) found that cover crops: *Secale* and *Brachypodium* improved infiltration rates by almost twice compared to conventional tillage (Ruiz-Colmenero *et al.* 2013). The study confirmed that steady-state infiltration was improved by both the increase in vertical pores (meso- and macro-) and by the absence of larger pore destruction – as had occurred in treatments of conventional tillage (Chisci *et al.* 2001; Ruiz-Colmenero *et al.* 2013). Here we note some the multifaceted co-benefits of cover crops and SOC in vineyards – both in terms of improving soil stability, preventing lateral run-off and increasing infiltration rate.

2.5 Conclusion

This review has explored the potential for vineyard floor management practice to significantly influence soil organic carbon concentrations, composition and turnover in vineyards of distinct locality and terroir. Cover crop use has proven beneficial both in the *retention* of SOC by preventing erosion and the *increase* in SOC via shoot-root C-translocation. Several studies in Spain, Italy, USA and Australia tested the influence of several different treatments of cover crops versus traditional practices (tillage or herbicide). Cover crop use on sloping vineyards has been effective in mitigating erosion through root-stabilisation. Moreover, cover crops have been shown to increase SOC stocks and concentrations in the mid-row and under-vine by up to 2.4 times. The additional use of composts, manure, mulch and biochar have been shown to be effective, both in terms of SOC concentrations and the composition of SOC in vineyards. Composition of SOC (physical and chemical) appears to correlate with the dominant biomass entering the soil. Biomass with higher lignin content (namely vine canes) require longer to decompose than low-lignin materials; however, can increase SOC concentrations to a level approximately the same as cow manure but at a far slower rate. Moreover, SOC composition and concentration were linked with microbial activity and abundance. Those soils with greater reserves of SOC provide an increased metabolic substrate for microbial activity, as evidenced through incubation studies that used soils from under different treatments of under-vine management. Specificity, as always, is pivotal to implementing the correct management strategy to suit both the desired outcome and the specific site variables in place. Grass cover crops can contribute greater SOC concentrations but require greater soil N; therefore, these may benefit from being grown in combination with a leguminous cover crop, which can provide the requisite N. Finally, the use of cover crops can facilitate numerous co-benefits to vineyard soil dynamics. Namely, they have been shown to improve soil stability – both at a micro-aggregate level (by SOC increases) and at the macro-aggregate level (by root stabilising). Improved infiltration and plant available water capacity can help to balance cover crop water use, while reducing run-off and surface evaporation.

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Chapter Three: Cover Crops and SOC Dynamics Under-Vine.

3 Cover crops and carbon stocks: How under-vineyard management influences SOC inputs and turnover in two vineyard

Manual soil coring to 30 cm can be hellish…

Sampling for the following manuscript left me exhausted, run-down and sick.

One day in a group meeting, the left side of my face became paralysed.

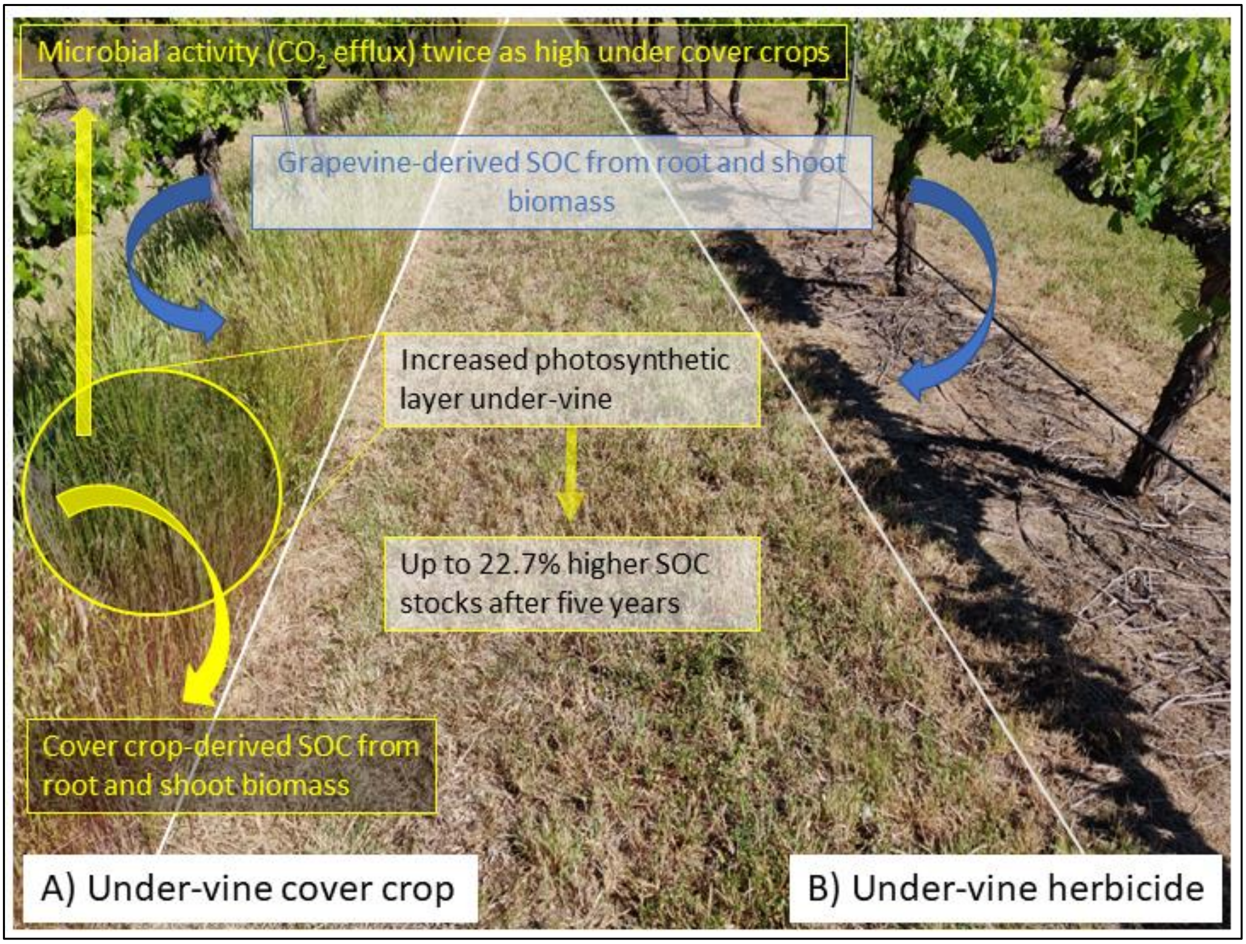
Dribbling and somewhat surprised, I asked my medico partner to make a swift diagnosis…

"Take a selfie with your eyebrows raised so we can rule out stroke" she said….

"…Yeah it's fine, it's just Bell's palsy…"

This is my first scientific publication, and I am proud.

Suffice to say, it cost me…



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Contribution to the Paper	Conceptualisation, data retrieval, data analysis, knowledge, drafting & editing.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/04/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Thomas Lines		
Contribution to the Paper	Conceptualisation, data retrieval, data analysis, knowledge, drafting & editing.		
Signature		Date	21/04/2022

Name of Co-Author	Christopher Penfold		
Contribution to the Paper	Conceptualisation, knowledge, drafting & editing.		
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Name of Co-Author	Professor Timothy Cavagnaro		
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Cover crops and carbon stocks: How under-vine management influences SOC inputs and turnover in two vineyards

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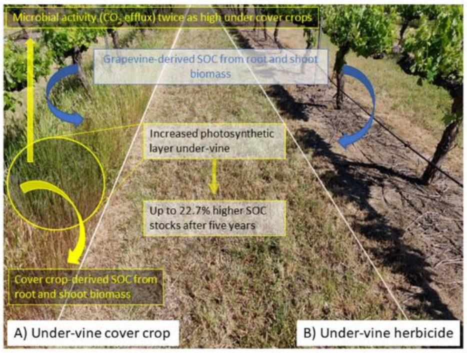
HIGHLIGHTS

- Soil organic carbon stocks differed significantly between under-vine treatments
- Soil organic carbon stocks were up to 22.7% higher under cover crop treatments
- Soil microbial respiration was more than twice as high in soil sampled from under both cover crop treatments
- Soil carbon turnover rates differed significantly between treatments and showed increased labile and resistant carbon in both cover crops
- Planting a cover crop under-vine has the ability to increase soil organic carbon stocks and influence turnover rates

GRAPHICAL ABSTRACT



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Sustainable viticulture

ABSTRACT

There is a growing awareness surrounding the importance of maintaining and increasing soil organic carbon (SOC, henceforth) stocks in vineyard systems. Increasing SOC positively influences numerous soil properties and has the added advantage of removing atmospheric CO_2, thereby helping to mitigate the effects of climate change. Cover crops have long been used to influence soil properties in vineyard mid-rows, including increasing SOC content. Few studies, however, have quantified cover crop influence on SOC stocks and composition in the under-vine area, owing to a general reluctance to adopt under-vine cover crop management. This research aims to quantify SOC stocks and dissolved organic carbon (DOC) in soils from four treatments of under-vine management practice including two cover crop combinations, a straw mulch and herbicide-managed control across two vineyard sites established in 2014. We sampled soils under-vine to depths of 0–30 cm (stratified; 0–10 cm and 10–30 cm) and quantified both SOC concentrations and bulk density to ascertain SOC stocks. Further to this, we quantified water extractable organic carbon (WEOC) as a measure of the labile carbon stock, and measured heterotrophic respiration in a laboratory incubation as an indication of SOC turnover. We found that cover crop-managed soil under-vine sequesters up to 23% more soil organic carbon (SOC) as the traditional, herbicide practice over a five-year period of growth. Microbial activity increased by more than double in cover crop soils, owing to an increase in DOC and that there is evidence for more resistant C in cover crop soils. These results suggest that cover crop management under-vine is a viable solution to increase SOC stocks within vineyard systems. Taken together, the results of this study indicate that a shift from bare earth to cover crops in the under-vine region has the potential to sequester carbon in vineyard soils.

1. Introduction

It is a rare and promising intersect, that which describes the multifarious benefits of sequestering soil organic carbon (SOC). This is the

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meeting of two oft-opposing ideologues – that of economic prosperity and that of environmental sustainability. The influences of SOC in agroecosystems are quantifiably numerous: enhancing soil structure (micro- and macro-aggregates); improving infiltration and water holding capacity; increasing cation exchangeability; and preventing topsoil loss, to name but a few (Brady, 2008; Paul, 2014). The improvement of infiltration and erosion-prevention by cover crops is well noted (Gulick et al., 1994; Ruiz-Colmenero et al., 2013). Cover crop root systems create vertical pores, improving rate of infiltration and stabilising organic carbon-rich topsoil, while above-ground biomass reduces the dispersive impact of raindrops (Novara et al., 2019; Ruiz-Colmenero et al., 2013). Furthermore, we are entering a novel space with the advent of carbon markets – policy framed toward the gradual mitigation of climate change through atmospheric carbon sequestration (Eldon and Gershenson, 2015). Vineyards can be viewed as ideal agroecosystems for SOC accumulation, owing to their large area (Brunori et al., 2016; Payen et al., 2020). In a recent report published by Wine Australia, it was found that Australian vineyards covered a total area of 146,244 ha, of which an estimated 79,262 ha (or ~ 52%) was located in South Australia (Milton et al., 2019). The sowing of cover crops in the mid-row is an often-utilised management practice, increasing photosynthesis and carbon storage (Steenwerth and Belina, 2008; Tesic et al., 2007).

Research into *under-vine* cover crop use is less expansive; however, increasing vineyard floor cover offers a greater potential for SOC increase versus a bare-earth practice (Novara et al., 2019; Ruiz-Colmenero et al., 2013). The paucity of data on under-vine cover crop use is likely related to a general hesitancy by viticulturists to adopt this style of management (Ball et al., 2020). This appears to stem from the perception that planting a cover crop in close proximity to grapevines could increase competition for key nutrients and water, leading to a yield penalty and impeding berry quality (Celette et al., 2009; Muscas et al., 2017). Several contrasting studies report both positive and negative impacts on yield and quality, with key factors being cover crop type and vineyard abiotic conditions (Karl et al., 2016; Peregrina et al., 2010). One study in north eastern USA found that a mixed grass sward did not affect berry yield or sugar but reduced acidity and increased berry tannin and phenolic components (Monteiro and Lopes, 2007). Another study in Chile found that the introduction of leguminous cover crops (or combinations of such) increased soil N to such an extent that they can supply grapevines with up to 40 kg N ha^{-1} (Ovalle et al., 2010). We may therefore conclude that specificity – both in terms of abiotic vineyard variables and cover crop type – appears to be the dominant driving forces behind the aforementioned observed outcomes and, thus, the adoption rate of alternative under-vine management practices.

Research on the influence of cover crops has a dual functionality; one of benefit and one of necessity. While the benefits are numerous (see above and further), there is also a necessity to explore vineyard management in the absence of herbicides, given the mounting pressure on their use in industry and indeed their banning in several jurisdictions (Beckie et al., 2020; Walsh and Kingwell, 2021). Several studies have sought to quantify the influence of under-vine management practices on SOC dynamics. The mechanistic approach to these studies appears driven by endemic issues associated with specific vineyard characteristics. Trials conducted in Spain and Italy on sloping vineyards are generally concerned with the *retention* of SOC – preventing erosion by using cover crop roots to anchor and stabilise soil (Novara et al., 2019; Ruiz-Colmenero et al., 2013). Other studies have adopted an SOC *increase* model, hypothesising increased SOC concentrations through atmospheric CO_2 capture, root translocation and litter deposition (Ball et al., 2020; Peregrina et al., 2014). Few trials have engaged more than one distinct vineyard type and between multiple distinct management practices (e.g. herbicide control, mulch addition, and growing two cover crops of different types). Fewer still have sought to quantify SOC *and* DOC (dissolved organic carbon) stocks to 0–30 cm with depth stratification, with the notable exception of a study completed in La Rioja region of central Spain (Peregrina et al., 2010).

If we are to understand the various elements of C sequestration under-vine, then it is also necessary to measure and analyse C dynamics under specific treatments, thus quantifying various C cycling processes. Previous research conducted by Ball et al. (2020) on the same vineyard sites focussed on SOC and nitrogen concentrations at 0–10 cm depth under grass/legume treatments, as well as mid-infrared spectroscopy (MIR) to model fine and coarse physicochemical fractions (Ball et al., 2020). Such research provides important insights into SOC dynamics under-vine, while also prompting further questions to be answered surrounding labile C and C turnover (Fleishman et al., 2021). Specifically, quantifying C stocks allows vineyard managers to make informed decisions about floor management. Moreover, quantifying labile C and microbial activity provides important insights into C turnover rates while, filling an important gap in under-vine research.

Here we present results of a study in which we sought to address three aims: 1) to quantify SOC stocks in soil sampled from beneath four under-vine treatments across two sites; 2) to quantify labile C in the form of DOC stocks from under the same treatments and; 3) to understand SOC turnover by measuring heterotrophic respiration dynamics.

2. Materials and methods

Soil carbon stocks and cycling, were assessed in soils collected from two under-vine vineyard floor management trials. At both trial sites, seven under-vine treatments were established in 2014, including five cover crops, a surface mulch and a herbicide-managed control (Penfold et al., 2018). The present study focused on four of the seven under-vine treatments, namely: *Lolium* and *Medicago* grown under-vine (medic and rye grass or M + R, hereafter); *Festuca* x *Trifolium* grown under-vine (fescue x clover or F + C, hereafter); Triticale mulch applied under-vine (TM, hereafter) and; a plant-free herbicide-managed, under-vine control (herbicide control or HC, hereafter). See Table 1 for detailed treatment descriptions. The first trial site was established 12th May 2014 at a vineyard in the Barossa Valley (BV, hereafter) wine region, near the town of Nuriootpa (34.477”S, 139.005″E). The second trial site was established 18th May 2014 at a vineyard site in the Langhorne Creek (LC, hereafter) wine region, south east of Strathalbyn (35.302”S, 138.952″E), both in South Australia. Both vineyard sites are situated on level ground, with minimal to no sloping gradient. See Table 2 for specific vineyard management details. The trial at both sites were arranged as a randomised complete block design (RCBD), with each of the four blocks comprising two rows of vines and arranged linearly (i.e. $n = 4$ blocks per vineyard). Each under-vine treatment was then randomly assigned to appear once within each block. Each treatment plot was comprised of nine vines (i.e. three panels), with a three-vine buffer zone (one panel) between treatments and also at the border of the trial zone to minimise edge effects.

Under vine cover crop composition and dry weight was estimated two times per year using the Botanal method (Tothill et al., 1992). For each plot, 6 × 0.25 m^2 quadrats were used, through visual assessment, to evaluate the relative species composition and quantity of the plant biomass. To ascertain biomass production, a curve of dry weights was produced from oven dried quadrat cuts which equated to 10 levels of production from zero to the maximum amount.

It should be noted that when the trials were established in 2014, SOC analysis under-vine was not a primary aim of the research team. Baseline data on SOC concentrations to a depth of 10 cm was recorded at both vineyard sites; however, no data exists to a depth of 10–30 cm, nor are there data on bulk density (SOC stocks). In spite on this, we are able to make comparisons between SOC concentrations (0–10 cm) prior to treatment establishment (2014 baseline data) and those data collected in this current study (2019 treatment data). These data will be presented in the Results section and discussed accordingly. Regardless of this paucity of baseline data, it should be noted that this is a treatment-based study, that is, a comparison between under-vine management practices, utilising a negative (herbicide-managed) control to understand differences in SOC and DOC stocks.

Table 1
List of four experimental treatments with common/cultivar name, species name and seeding rates as applied when the trial commenced in 2014. Additionally, 2019 botonal (cover crop composition data) data is listed for across both sites, including residue C:N ratios (where applicable). Botonal data shows relative mass of each cover crop within its combination (kg ha $^{-1}$).

Treatment	Herbicide control	Triticale mulch	Medic/ryegrass	Fescue/clover
Abbreviated code	HC	TM	M + R	F + C
Cultivar name	NA	NA	Safeguard ryegrass / Scimitar	Sheep fescue / Strawberry clover
Species name	NA	NA	*Lolium rigidum / Medicago polymorpha*	Festuca sp. / *Trifolium fragiferum*
Seeding/application rate	Glyphosate 540 (3 L ha $^{-1}$)	50 t ha $^{-1}$	M: 20 / R: 30 kg ha $^{-1}$	F: 3 / C: 20 kg ha $^{-1}$
Plant description			Annual self-regenerating pasture legume and grass	Perennial legume and grass
Botanal data (kg ha $^{-1}$)				
- **Langhorne Creek**	NA	NA	M: 1354 / R: 1311	F: 4032 / C: 0
- **Barossa Valley**	NA	NA	M: 912 / R: 1499	F: 456 / C: 96

2.1. Soil sampling, processing and bulk density

Soil sampling at both trial sites was conducted to coincide with grapevine bud burst (October 2019, austral spring). From each treatment plot, triplicate soil samples were collected from a 0–30 cm soil layer using a stainless steel split core and slide hammer (length: 30 cm, diameter: 5 cm). These soil cores were separated into two depths (0–10 cm and 10–30 cm), and the triplicate samples for each depth pooled at the plot level. Thus for each plot there was one soil samples from the 0–10 cm layer, and another from the 10–30 cm layer, both of which were composites of the three within-plot cores. With four treatments, and four blocks, this produced a total of 16 samples per depth, or 32 samples from each of the two sites. Each sample (treatment and depth) was weighed fresh, before being air-dried at 40 °C for one week (to preserve organic C) and sieved to <2 mm with aggregates gently crushed to avoid grinding any plant-derived organic matter. Soil mass was recorded at each stage of drying. Post-sieving, soil samples of size fraction <2 mm were weighed (bulk) and then subsampled using a riffle box (CiviLab Australia). One subsample was weighed and preserved for SOC analysis, while a second subsample was analysed for gravimetric water content (GWC) by oven-drying at 105 °C for 48-h (Walter et al., 2016). The >2 mm size fraction (gravel content) were also analysed for GWC and total rock density to allow for gravel content corrections. Rock volume was calculated using the water displacement method (Cunningham et al., 2012), with a standard curve then calculated to reflect the displaced volume of water (mL) vs rock mass (g), with the resultant slope and intercept used to calculate rock volume/soil core (R^2 correlation was 0.999). Thus, we were able to back-calculate total GWC of the initial fresh samples and accurately account for bulk density for each treatment/depth, while taking into account necessary correction factors for gravel content within samples (Walter et al., 2016).

Table 2
List of grapevine and vineyard variables for Langhorne Creek (LC) and Barossa Valley (BV) sites, including management vine and mid-row management practices.

Site and properties	Langhorne Creek	Barossa Valley
Soil classification[a]	Brown Sodosol	Brown Sodosol
Grape varietal	Cabernet Sauvignon	Shiraz
Vine age	21 years	19 years
Rootstock type	Own (Cab LC-10)	Own (Shiraz and SARDI)
Training method	Single cordon trellis	Single wire cordon
Pruning method	Saw box pruned (15–18 t ha $^{-1}$)	Two-bud spur-pruned (~20 buds per vine)
Pest/disease management	Occasional foliar fungicide	Occasional foliar fungicide
Mid-row management	Slashing and heavy rolling	Triticale mown
Rooting depth	~300–400 cm	1.5–3 m
Irrigation regime	3.5 ML ha $^{-1}$	1 ML ha $^{-1}$
Dripper spacing	2.5 m row $^{-1}$	9.1 m row $^{-1}$
Elevation above sea level	31 m	277 m
Mean annual precipitation (2019)	264 mm	296 mm

[a] Data obtained from Department of Environment and Water Soil and Land Program (Hall et al., 2009).

2.2. Soil organic carbon analysis

Soil organic carbon (SOC) concentrations were analysed on the <2 mm size fraction, with a representative subsample selected for analysis using a riffle box. Total C analysis was conducted by the Australian Precision Agricultural Laboratory (APAL) by LECO dry combustion method. Samples were pre-tested for the presence of inorganic carbonates ($CaCO_3$) using hydrochloric acid (fizz test). Total organic carbon (TOC) was measured by wet combustion (Walkley and Black method, Thermo Fischer Scientific, USA) (Rayment and Lyons, 2011). In addition to SOC, total nitrogen (TN), nitrate (NO_3^-) and ammonium (NH_4^+) were also analysed by dry combustion and, as well as soil $EC_{1:5\ soil:water}$ and $pH_{1:5\ soil:water}$ (see Tables 3 and 4) (Rayment and Lyons, 2011). Organic carbon data was presented as % SOC, which was converted to SOC concentration (g kg $^{-1}$) and multiplied by the sample bulk density and depth (cm) to ascertain an SOC stock (t C ha $^{-1}$). In addition to quantifying TOC concentrations and stocks, we also analysed for labile carbon in the form of dissolved organic carbon (DOC). Processing of samples for DOC was conducted using the water extractable carbon method (WEOC) as follows: Subsamples (3 g) of soil were diluted with 30 mL of reverse osmosis water (10:1 dilution) and shaken using a reciprocating shaker (RATEK) for 1 h at 100 RPM (Akhtar et al., 2016). Samples were then centrifuged for 10 min at 5000 x G, before 20 mL of supernatant was collected and refrigerated prior to analysis (Stirling et al., 2019). Samples were then analysed for DOC by Envirolab Services Pty Ltd. (Perth, Western Australia) using the dry combustion method (Analytik Gena).

2.3. Soil respiration analysis

Prior to incubation and respiration analysis, soil samples were measured for water holding capacity (WHC) using the hanging water column method, as described by Cavagnaro (2016). There were no significant differences in WHC between soil treatments ($p > 0.05$), therefore WHC (g soil / g water) was averaged between treatments (per site). We determined that samples from depth 0–10 cm were most suitable for respiration analysis owing to their significant ($p < 0.05$) differences in DOC stocks (see Results). Soil samples of 50 g (air-dried and adjusted for GWC) were weighed into 250 mL incubation jars (Nalgene, 250 mL) and brought up to 60% of WHC (Liu et al., 2019; Stirling et al., 2019). Incubation jars were kept in temperature and humidity controlled incubation ovens at a constant temperature of 25 °C. Soil respiration was analysed using an LI-820 CO_2 gas analyser (LiCor, NC) at hours 0, 6 and 30 (to allow for initial inorganic CO_2 flux) and at hours 53, 102, 150, 220, 314 and 440 (Stirling et al., 2019). Prior to CO_2 analysis, headspace was flushed with air (of a known CO_2 concentration) to remove residual CO_2 from the headspace, before being sealed and incubated at room temperature for 30 min.

Table 3

Physicochemical properties of soils collected from beneath treatments (0–10 cm and 10–30 cm depth) at LC and BC vineyard sites. Treatments are: HC (herbicide control), TM (Triticale mulch), M + R (medic/ryegrass) and F + C (fescue/clover). N.B the experimental treatments at the site are described in Table 1. Values are Mean ± SE.

Site	HC (0–10 cm)		TM (0–10 cm)		M + R (0–10 cm)		F + C (0–10 cm)		HC (10–30 cm)		TM (10–30 cm)		M + R (10–30 cm)		F + C (10–30 cm)	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Langhorne Creek																
Total nitrogen (%)	0.19	0.00	0.20	0.01	0.21	0.01	0.22	0.01	0.15	0.00	0.14	0.01	0.16	0.00	0.16	0.01
Total nitrate (mg kg^{-1})	7.30	1.92	3.13	0.86	7.10	3.90	5.25	2.03	1.43	0.23	1.40	–	1.03	0.03	1.93	0.58
Total ammonium (mg kg^{-1})	1.75	0.22	1.78	0.23	2.00	0.30	–	–	1.15	0.15	1.40	0.40	1.20	0.10	1.60	0.06
C:N ratio	7:1	–	6:1	–	7:1	–	5:1	–	4:1	–	4:1	–	4:1	–	3:1	–
pH 1:5 Cl	7.46	0.21	7.20	0.12	6.91	0.19	7.07	0.05	7.16	0.07	7.04	0.16	7.21	0.09	7.54	0.14
EC 1:5 dS m^{-1}	0.25	0.05	0.24	0.07	0.42	0.14	0.41	0.13	0.16	0.02	0.14	0.03	0.30	0.06	0.29	0.06
Sand (%)	65.75	0.75	66.50	2.10	66.50	0.87	67.50	1.32	54.00	2.97	61.50	2.53	58.50	1.50	54.25	1.25
Silt (%)	25.5	0.65	24.50	1.44	24.50	0.50	24.25	0.85	27.50	0.50	23.75	0.75	26.75	0.48	26.75	0.25
Clay (%)	8.55	0.36	9.05	0.71	8.93	0.71	8.58	0.53	18.75	2.72	14.65	2.56	15.00	1.73	19.25	1.03
Barossa Valley																
Total nitrogen (%)	0.18	0.01	0.21	0.01	0.22	0.01	0.19	0.01	0.15	0.00	0.17	0.00	0.16	0.01	0.16	0.00
Total nitrate (mg kg^{-1})	3.20	0.93	2.23	0.81	N/A	N/A	9.43	3.72	1.88	0.24	1.10	–	1.20	–	1.65	0.18
Total ammonium (mg kg^{-1})	2.23	0.30	2.43	0.24	1.68	0.17	1.27	0.13	4.10	2.10	2.45	1.25	1.55	0.23	2.00	0.46
C:N ratio	6:1	–	7:1	–	7:1	–	6:1	–	4:1	–	4:1	–	4:1	–	3:1	–
pH 1:5 Cl	6.44	0.13	6.80	0.05	6.78	0.15	6.81	0.06	6.47	0.09	6.85	0.08	6.81	0.09	6.75	0.15
EC 1:5 dS m^{-1}	0.08	0.01	0.11	0.01	0.11	0.02	0.18	0.03	0.07	0.01	0.09	0.01	0.10	0.02	0.08	0.00
Sand (%)	76.50	3.43	74.75	2.02	74.25	1.55	73.25	2.39	70.50	1.94	65.25	3.50	63.75	5.65	66.25	5.17
Silt (%)	16.00	2.80	17.75	1.75	17.25	1.65	18.75	2.02	18.50	1.85	20.25	1.49	21.25	1.03	19.75	1.25
Clay (%)	7.68	0.61	7.70	0.23	8.45	0.41	8.13	0.66	10.65	0.35	14.65	3.20	15.28	4.67	14.15	3.85

2.4. Data analyses

Prior to significance testing all treatment variables were tested for normality and homoscedasticity using the Wilkes-Shapiro test. Those data that were found to be non-normal in distribution were transformed using a log, square root, cube or exponent transformation. Significance testing of soil organic carbon (SOC) and dissolved organic carbon (DOC) stocks was conducted by two-way analysis of variance (ANOVA), using *treatment* and *block* as factors in the analysis. This was conducted in accordance with the randomised complete block design (RCBD) layout of the vineyard trial sites as a means of accounting for any unknown spatial heterogeneity at the site level. Those ANOVAs that were significant ($p < 0.05$) underwent further post hoc analysis (Tukey's HSD) to reveal significant differences between treatment means ($\alpha < 0.05$). Boxplots were created to visualise pairwise treatment differences, with *p*-values attached and different letters denoting significantly different treatments. All data were analysed using R open source statistical software (version 1.2.5033) (Team, 2013), with additional software package "agricolae", alpha was set at 0.05 (De Mendiburu, 2014).

Respiration data were processed stepwise, with initial CO_2 output (ppm) summed for each treatment peak and converted to account for mass of soil (C-CO_2. kg soil^{-1}. hr^{-1}), and lastly converted to cumulative respiration per treatment replicate. Data analysis and visualisation was conducted in three parts, with initial one-way ANOVAs used to test significant

Table 4

Average carbon, bulk density and gravimetric water content measurements of soils collected from beneath treatments (0–10 cm and 10–30 cm depth) at LC and BV vineyard sites. Treatment variables with different subscript letters are significantly different from each other ($p < 0.01$), after Tukey's HSD post-hoc analysis. Treatments are: HC (herbicide control), TM (Triticale mulch), M + R (medic/ryegrass) and F + C (fescue/clover). N·B the experimental treatments at the site are described in Table 1. Values are Mean ± SE.

Site	HC (0–10 cm)		TM (0–10 cm)		M + R (0–10 cm)		F + C (0–10 cm)		HC (10–30 cm)		TM (10–30 cm)		M + R (10–30 cm)		F + C (10–30 cm)	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Langhorne Creek																
Total carbon (g kg^{-1})	9.15	0.34	13.83	2.24	13.20	2.24	15.88	1.31	4.75	0.69	6.30	0.63	6.70	0.76	6.18	0.19
Total organic carbon (g kg^{-1})	9.15	0.34	13.33	2.48	11.83	1.01	14.23	1.18	4.75	0.69	5.28	1.21	5.78	0.46	5.68	0.38
Total dissolved carbon (g kg^{-1})	0.10 b	0.01	0.12 b	0.02	0.14 ab	0.01	0.20 a	0.01	0.13	0.02	0.11	0.02	0.13	0.02	0.16	0.01
Total CO_2 (mg kg^{-1} h^{-1})	825.56 b	182.95	960.25 ab	95.25	1178.95 ab	318.21	1370.38 a	123.70	NA	NA	NA	NA	NA	NA	NA	NA
Bulk density (g cm^3)	1.42	0.03	1.31	0.03	1.43	0.17	1.25	0.05	1.44	0.03	1.40	0.02	1.35	0.05	1.40	0.04
Gravimetric water content (%)	9.9 ab	1.8	15.6 a	1.0	7.9 b	1.1	9.9 ab	1.1	13.7 ab	1.5	15.2 a	0.5	9.8 b	0.7	11.9 ab	0.9
Barossa Valley																
Total carbon (g kg^{-1})	10.38	0.44	11.53	0.46	14.13	0.63	15.10	1.32	5.00	0.24	5.28	0.05	6.65	0.49	6.15	0.78
Total organic carbon (g kg^{-1})	10.38	0.44	11.53	0.46	14.13	0.63	15.10	1.32	5.00 b	0.24	5.28 b	0.05	5.65 a	0.78	6.15 ab	0.78
Total dissolved carbon (g kg^{-1})	0.13	0.01	0.14	0.00	0.16	0.01	0.15	0.01	0.19	0.02	0.16	0.03	0.20	0.05	0.17	0.02
Total CO_2 (mg kg^{-1} h^{-1})	463.27 b	48.87	723.89 b	105.69	1197.34 a	258.21	1228.88 a	360.51	NA	NA	NA	NA	NA	NA	NA	NA
Bulk density (g cm^3)	1.28	0.09	1.36	0.04	1.26	0.01	1.29	0.03	1.48	0.04	1.44	0.04	1.46	0.05	1.44	0.03
Gravimetric water content (%)	5.1 ab	0.5	7.9 a	0.4	4.7 b	0.3	4.5 ab	0.4	8.2	0.2	10.3	0.8	7.8	2.3	7.5	2.0

differences in total cumulative CO_2 at the final time point (440 h). Those data that showed significance ($p < 0.05$) underwent further post hoc analysis, with data visualised in side-by-side boxplots, with different letters used to denote significantly different treatments (Tukey HSD, $\alpha < 0.05$). Cumulative respiration curves were modelled using the monomolecular (aka Mitscherlich) growth function (Eq. (1)) (Paine et al., 2012) using R-based non-linear mixed-effect model package "mosaic" with the 'algorithm' set to 'port' (Pruim et al., 2017).

$$Resp = \frac{(K - M_0)}{(K^{rt})} + bt \qquad (1)$$

where M_0 represents the cumulative respiration (mg CO_2. kg soil^{-1}. hr^{-1}) at time = 0, K represents CO_2 respiration as it approaches the asymptote of growth, r represents the decay constant and b represents any linear growth beyond the asymptote.

Significance testing was then conducted on each of the four model parameters (as well as the initial slope) using one-way ANOVA. Tukey's post-hoc analysis was then used to discern which treatment parameters were significantly different from each other. Cumulative growth curves were visualised using R package "ggplot2", with the geom_line function highlighting 95% confidence intervals (Wickham, 2009).

3. Results

As previously noted, baseline (2014 pre-trial) SOC data was collected; however only to a depth of 0–10 cm and was not accompanied by bulk density data. As such, we can only compare 2019 SOC concentrations with 2014 baseline data at this depth. Data form 2014 was averaged across each site. In order to make an accurate treatment-based comparison from the baseline, those data from 2019 were treated stepwise: Firstly, 2019 SOC cover crop data were averaged and recorded, then 2019 HC (control) data were averaged and deducted from cover crop data, thus controlling for SOC increase as a function of time, rather than a specific treatment-based difference. Baseline (2014) SOC concentrations at 0–10 cm were as follows: Langhorne Creek (LC) = 0.92 mg kg^{-1} and Barossa Valley (BV) = 0.75 mg kg^{-1}. Cover crop data collected from 2019 are as follows: LC = 1.72 mg kg^{-1} and BV = 1.77 mg kg^{-1}. Accounting for baseline SOC increase as a function of time, we can report a treatment-based (cover crop vs control) increase in SOC concentrations at 0–10 cm of 17.8% at LC and 13.6% at BV.

3.1. Carbon stocks

Carbon stock data were stratified into three (depth) groups for statistical analysis, namely: 0–10 cm, 10–30 cm and 0–30 cm (combined total). An average of both cover crop treatments vs the bare earth control revealed an increase in SOC stocks of 17.2% and 22.7% at LC and BV respectively at a depth 0–30 cm. At the BV site, SOC stocks at 10–30 cm (see Fig. 1) and 0–30 cm (see Fig. 3) showed significant differences ($p < 0.01$ and $p < 0.01$ respectively) with both the herbicide control and straw mulch treatments significantly lower than either of the cover crop treatments (M + R presented the highest SOC stock overall). Soil organic carbon stocks at the LC site presented no significant differences at the treatment or block level, however, at 0–10 cm the control treatment appeared to represent the lowest SOC stock, with a clear trend visible (see Fig. 1). Indeed, at both sites SOC (0–30 cm total stocks) showed a treatment-based pattern

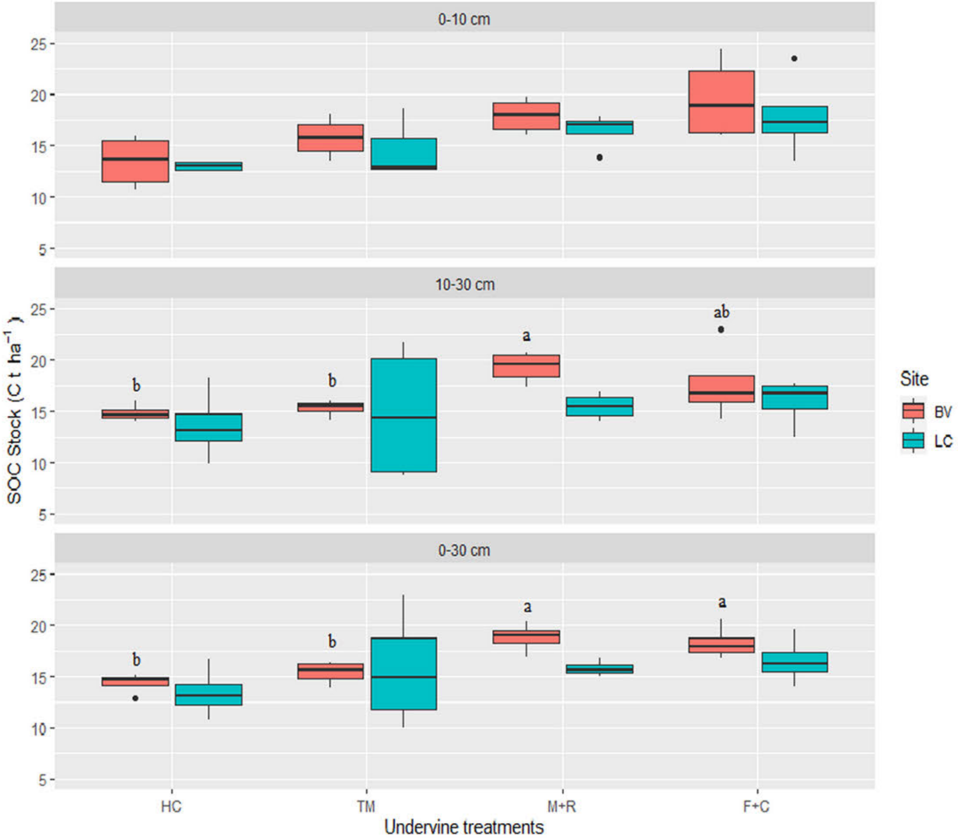

Fig. 1. Boxplots showing median values for SOC stocks (C t ha^{-1}) at depths of 0–10 cm, 10–30 cm and 0–30 cm (total) in soil sampled from beneath under-vine treatments of herbicide control (HC), Triticale mulch (TM), medic-rye (M + R) and fescue-clover (F + C) at sites BV and LC. Letters above treatments (Tukey post-hoc testing) denote significantly different treatments ($p < 0.01$), while those treatments without letters are not significant ($p > 0.05$).

of increase in the order of HC < TM < M + R and F + C (patterns were statistically significant at BV, though not at LC).

Dissolved organic carbon (DOC) stocks presented a similar pattern of high variability at both sites, with increased variability at 10–30 cm depths. Dissolved organic carbon stocks were only significantly different ($p < 0.01$) at the LC site (0–10 cm), with both cover crop soils showing significantly higher DOC stocks than both the HC (control) and TM (mulch), with M + R not significantly different to either, likely owing to a higher degree of variability within treatment (see Fig. 2). Both soil organic carbon (SOC) and dissolved organic carbon (DOC) stocks showed a high degree of variability (within treatment) at depths of both 0–10 cm and 10–30 cm. Certain under-vine treatments (e.g. M + R) showed less variability at 0–10 cm than at 10–30 cm, however, the straw mulch treatment showed high variability throughout (see Figs. 1 and 2). Gravimetric water content (GWC) measurements maintained a distinct treatment-based pattern and was significantly different ($p < 0.01$) between all treatments and depths at LC and at a depth of 0–10 cm at BV. In each case, the straw mulch (TM) showed the highest GWC, while cover crop treatments of M + R presented the lowest and F + C and HC (control) were not statistically different either.

3.2. Cumulative respiration

Cumulative respiration at the end of the experiment (440 h) differed significantly among treatments (see Fig. 4). Specifically, cumulative respiration in soils from LC was significantly higher ($p < 0.02$) in F + C treatment than the HC (control) treatment (see Fig. 4). For BV, cumulative respiration was significantly higher ($p < 0.01$) in both cover crop (F + C and M + R) treatments than in HC or TM. Monomolecular models were successfully fitted to the cumulative respiration data. In soils from the LC site, the decay constant (r) was significantly lower ($p < 0.02$) in the HC treatment than all other treatments and the linear growth rate beyond the asymptote (b) was highest for F + C ($p < 0.03$) (see Table 5 and Fig. 4). For the BV site, CO_2 respiration approaching the asymptote (M) was highest in medic/ryegrass treatments and lowest in the herbicide control ($p < 0.01$) and cumulative respiration (M_0) was also highest in M + R, while lowest in all other treatments ($p < 0.01$) (see Table 5 and Fig. 4). The initial slope of the growth rate was also highest in treatments of medic/ryegrass and lowest in the herbicide control ($p < 0.01$) (see Table 5).

4. Discussion

4.1. Carbon stocks increase under cover crops

A pattern of increased SOC was observed at both sites throughout most depths, although only significant ($p < 0.01$) differences were found in SOC stocks from BV (10–30 and 0–30 cm) and DOC stocks from LC (0–10 cm). The overall pattern appears to show an increase in SOC and, to a somewhat lesser extent, DOC stocks under cover crop treatments versus mulch and herbicide. The majority of studies conducted found evidence to suggest that cover-cropped soil was higher in SOC concentrations and stocks when compared to a negative control (traditional bare-earth practice or conventional tillage practice) (Ball et al., 2020; Novara et al., 2019; Ruiz-Colmenero et al., 2013). We suggest a distinct treatment-based (or management-based) approach to increasing SOC stocks, that is, the sowing of cover crops in place of herbicide-managed bare earth. This approach suggests a mechanistic benefit – that which increases SOC as a function of inputs such as: root and shoot biomass, root exudates and microbial

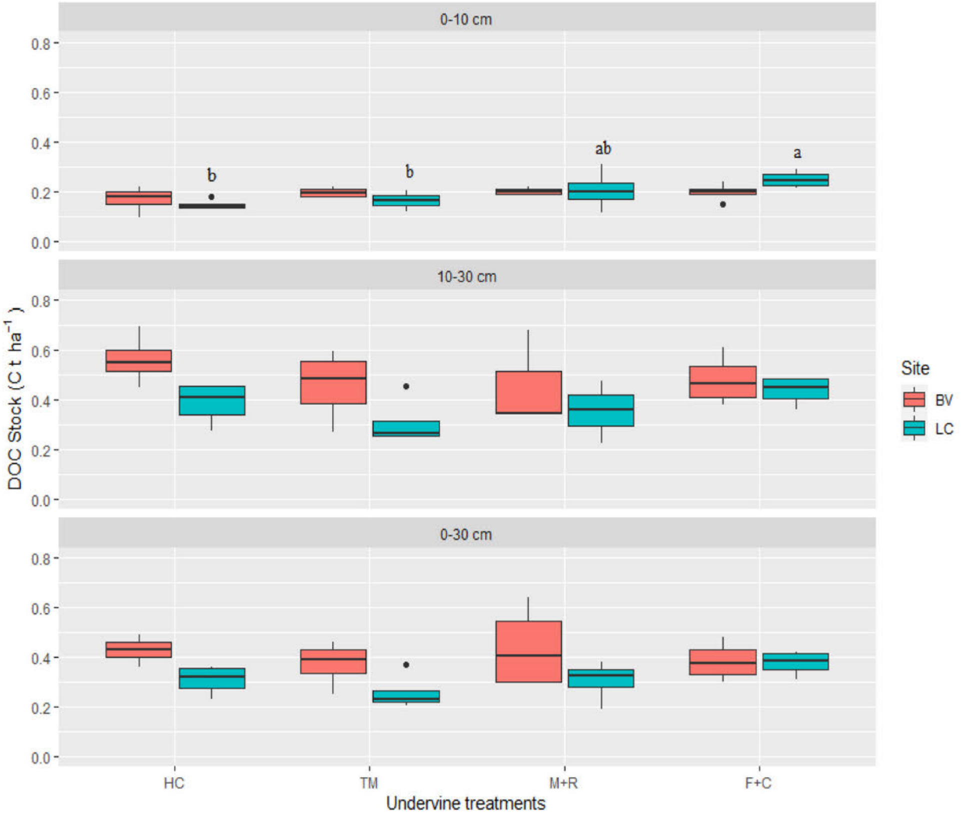

Fig. 2. Boxplots showing median values for DOC stocks (C t ha^{-1}) at depths of 0–10 cm, 10–30 cm and 0–30 cm (total) in soil sampled from beneath under-vine treatments of herbicide control (HC), Triticale mulch (TM), medic-rye (M + R) and fescue-clover (F + C) at sites LC and BV. Letters above treatments (Tukey post-hoc testing) denote significantly different treatments ($p < 0.01$), while those treatments without letters are not significant ($p > 0.05$).

Table 5

Monomolecular (aka Mitscherlich) growth model modified from (Paine et al., 2012), where M_0 represents the cumulative respiration (mg C-CO_2 kg soil^{-1}. hr^{-1}) at time = 0, K represents CO_2 respiration as it approaches the asymptote of growth, r represents the decay constant and b represents any linear growth beyond the asymptote. Treatment means are presented (n = 4) with relative p-values extracted from one-way analysis of variance. Significantly different means are represented with different letters (Tukey's HSD, α = 0.05).

Site	K ($p > 0.05$		r ($p < 0.02$)		M_0 ($p > 0.05$)		b ($p < 0.03$)		Initial slope ($p < 0.01$)	
Langhorne Creek										
M + R	799.125	–	0.007	ab	1.449	–	0.948	ab	6.532	a
F + C	898.132	–	0.008	ab	0.000	–	1.135	a	8.320	a
TM	644.615	–	0.009	a	1.854	–	0.819	ab	6.604	ab
HC	885.426	–	0.005	b	3.242	–	0.157	b	4.568	b
Barossa Valley	K ($p < 0.01$)		r ($p > 0.05$)		M_0 ($p < 0.02$)		b ($p > 0.05$)		Initial slope ($p < 0.03$)	
M + R	894.634	a	0.008	–	10.058	a	0.806	–	7.883	a
F + C	840.456	ab	0.008	–	2.937	b	1.042	–	7.742	ab
TM	424.880	ab	0.009	–	2.737	b	0.736	–	4.535	bc
HC	292.735	b	0.007	–	4.205	b	0.454	–	2.473	c

biomass as a function of atmospheric C fixation and translocation (Mutegi et al., 2011; Peregrina et al., 2014). In addition to these primary pathways, we may also speculate on several integrated, or cyclical, secondary pathways that build SOC. At the molecular level, C provides increased bonding sites for cation bridging and the formation of micro-aggregates (Clough and Skjemstad, 2000; Solly et al., 2020). Following this, macro-aggregates are formed and stabilised by fibrous cover crop roots and various organic secretions (Novara et al., 2019). In this, we may suggest that cover crop presence not only increases SOC but also improves aggregate stability and SOC retention within agroecosystems (Novara et al., 2019; Ruiz-Colmenero et al., 2013).

Above-ground plant biomass is a key generator of C fixation and translocation (Mutegi et al., 2011; Veloso et al., 2018). It therefore stands to reason that we observed greater SOC stocks under F + C treatments than under M + R, owing to a greater fescue biomass both above- and below-ground (Ball et al., 2020). This was particularly evident at LC, where increased rates of irrigation likely contributed to delayed fescue senescence, allowing for increased rates of photosynthesis and biomass accumulation. The disparity in soil moisture between sites as a function of irrigation regimes is evidenced by gravimetric water contents (see Table 4). Increased use of irrigation at LC not only halted senescence but also likely restricted above-ground biomass from decomposing and becoming incorporated into under-vine soil. The disparity in biomass was observed in Table 1, where above-ground biomass for medic and fescue at LC was 32.6% and 88.7% higher than that recorded at BV respectively. Lower irrigation rates encourage summer cover crop senescence and therefore a reduction in photosynthetic material across both cover crop treatments. Fescue grass cover appeared to be highly influenced by water availability; however, this was not the same for ryegrass biomass, which was observed to be 14.3% higher at BV than LC and thus relatively unaffected by reduced water availability (Table 1). These results accord well with previous studies that suggest fescue cover crops are prone to high water-uptake and therefore, perhaps most applicable in high rainfall climates where excess precipitation may be a problem (Celette et al., 2005). Therefore, we may suggest that in hot, dry (Mediterranean-type) climates, a ryegrass or ryegrass combination may be more appropriate where water is a precious resource.

Improved soil structure under nitrogen-fixing cover crops is well-documented (Peoples et al., 2015; Ramirez-Garcia et al., 2015). Thus, as well as increasing plant cover, we note the capacity of medic to fix nitrogen symbiotically with Rhizobium, as a key factor leading to increased SOC stocks in treatments of M + R (Peoples et al., 2015). The role of N in stabilising soil aggregates and thus accumulating SOC is well-cited, albeit without abundant evidence of the exact mechanistic pathways (Jastrow and Miller, 1996). Although the clover component of the "fescue + clover" treatment is also N-fixing, over time the more vigorous fescue grass has outcompeted the clover and become dominant, thus negating any residual nitrogen-fixing characteristic of the clover (see Table 1). Total soil N was measured prior to sampling; however, no significant differences were observed between under-vine soil treatments. This was likely due to the transient nature of N-species and the time of sampling – austral spring – when grapevine vegetative growth and budburst increased mineral N uptake, likely leading to depletion in under-vine soils (Keller, 2015). A previous study conducted on the same under-vine treatments found that both SOC and total N were higher under "grass + legume" cover crops than in the bare earth control (Ball et al., 2020). The authors suggest this was the result of leguminous N increasing grass root biomass, which has been shown to contribute ~35% more SOC than above-ground biomass (Puget and Drinkwater, 2001). In our own study, we found increased SOC under treatments of M + R (grass + legume) at BV – a result according with the aforementioned study, albeit without sufficient evidence for the same conclusion. We acknowledge; therefore, that in order to make firmer assertions on the role of leguminous cover crops (M + R) to influence total soil N – and therefore SOC dynamics – we would have to sample at seasonal time points to capture transient N concentrations.

We observed a clear pattern of difference in SOC stocks between treatments. However, this was less the case with DOC stocks (WEOC), where the only significantly different result ($p < 0.01$) was observed at LC (0–10 cm depth) and there were few clear visual patterns of difference. Moreover, DOC concentrations comprised only ~2% of the overall TOC concentrations between treatments, while the ratio of TOC:DOC did not differ significantly. Previous research suggests that soil moisture content (GWC) is a central factor in determining the fate of DOC within the soil profile, where soil treatments with higher GWC are more prone to DOC-leaching and therefore lower concentrations of solubilised C (Ponizovsky et al., 2006). Our own results showed significant differences in GWC ($p < 0.01$), with treatments of straw mulch (TM) showing the highest moisture content and M + R the lowest. These results are inversely proportional to those comparing DOC stocks, thus suggestive that increased moisture retention under straw mulch has led to decreased levels of DOC. These comparisons are true for the LC site but not the BV site, where irrigation regimes are less frequent and less intense.

The largely non-significant differences in DOC between treatments may suggest that the SOC composition picture is incomplete, with further physical and chemical fractionation required to understand the C pool composition. Previous work conducted on the same sites by Ball et al. (2020) used physical fractionation methods to measure SOC and total N under treatments of "grass", "grass + legume" and a "bare earth control". Their study found that SOC concentrations were significantly increased in the more stable fine fraction, especially in the "grass" treatment, owing to greater root mass (Ball et al., 2020; Puget and Drinkwater, 2001). The authors also measured SOC and total N concentrations in the coarse soil fraction, which is known to be more sensitive to change and thus perhaps a more accurate measure of short-term change in a system (Ball et al., 2020; Ojeda et al., 2018). Measuring DOC provided insight into the most labile (easily metabolised) form of SOC; however, we acknowledge that the story is incomplete. In this, we suggest future studies that may seek to dissect the various SOC pools (physical and chemical), thus characterising under-vine SOC change over time.

4.2. Soils breathe deeply under cover crops

As expected, both cumulative soil respiration and logistic growth parameters were significantly higher ($p < 0.01$) in soils sampled from under both cover crop treatments versus the straw mulch and higher still than the herbicide control. Soil respiration was measured partly as a proxy for microbial activity (and thus SOC via total accumulated CO_2, see Fig. 3) and partly as means of validating DOC content (as shown by logistic growth parameters, see Table 5 and Fig. 4). At both sites, total accumulated CO_2 was significantly higher ($p < 0.01$) in cover crop treatments, with little site-specific variation. Only at LC; however, did DOC content appear significantly higher under cover crop treatments. Several potential explanations for this disparity may be posited; however, each remains speculative and unsubstantiated within the confines of this research. One explanation may be that DOC pools are in such constant flux with the total organic carbon (TOC) pool that we may not be capturing significant differences in DOC at a single sample point (Kuzyakov, 2011). Perhaps a more likely explanation – at least in the confines of data collected – pertains to a point made in the previous section: that regarding unexplored SOC pools. We hypothesised that the more microbially-favourable DOC pool would correlate with increased respiration under-vine; however, this was not the case. Therefore, we suggest that microbial communities were able to access different SOC pools during incubation. This hypothesis appears most plausible in those soils sampled from BV, where logistic growth parameter *b* (linear growth beyond the asymptote), was both significant ($p < 0.01$) and showed a clear pattern of separation from other, non-cover crop treatments (see Fig. 4 and Table 5). Previous research conducted by Stirling et al. (2019) showed similar logistic growth parameters during the incubation of pine needle litters (low-quality litter comprised of relatively high lignin content). A 2013 review on organic matter cycling suggested that high quality litters (e.g. medic) initially decompose rapidly, due to abundant N; however, tend to slow in decomposition as N is depleted, leading to more stable accumulation of SOC by microbial pathways (Cotrufo et al., 2013). By contrast, low-quality litters (high C vs N content) tend to decompose slowly initially as microbial communities slowly "mine" N from more resistant C molecules, after which decomposition rates increase as easily-metabolised molecules become available (Cotrufo et al., 2013; Craine et al., 2007).

Clear differences in SOC and heterotrophic respiration were observed; however, these were not correlated with the measured DOC pool. While DOC was found to be higher in cover crop soils collected from LC, overall microbial respiration was found to be largely consistent between the two sites. Another explanation for this may concern site-specific irrigation practices: higher rates of irrigation at LC may increase the decomposition process, enhancing the dissolution of labile C and creating an observably higher DOC pool *in situ* (Cisneros-Dozal et al., 2007; Gmach et al., 2020). Again, although unsubstantiated, this hypothesis may be reasonable in light of respiration experiments, where soils from BV were incubated under the same %WHC as those from LC, thus normalising the availability of DOC (Gmach et al., 2020). This finding is important, with several studies asserting that fluxes between C pools or from the C source (plant biomass) are dynamic in nature and change rapidly depending on specific edaphic biotic and abiotic factors (Buchmann, 2000; Plante et al., 2010). Moreover, fluxes associated with microbially-favourable SOC are thought to be in constant flux not only with internal SOC pools, but also directly from their above- and below-ground litter sources (Kuzyakov, 2011).

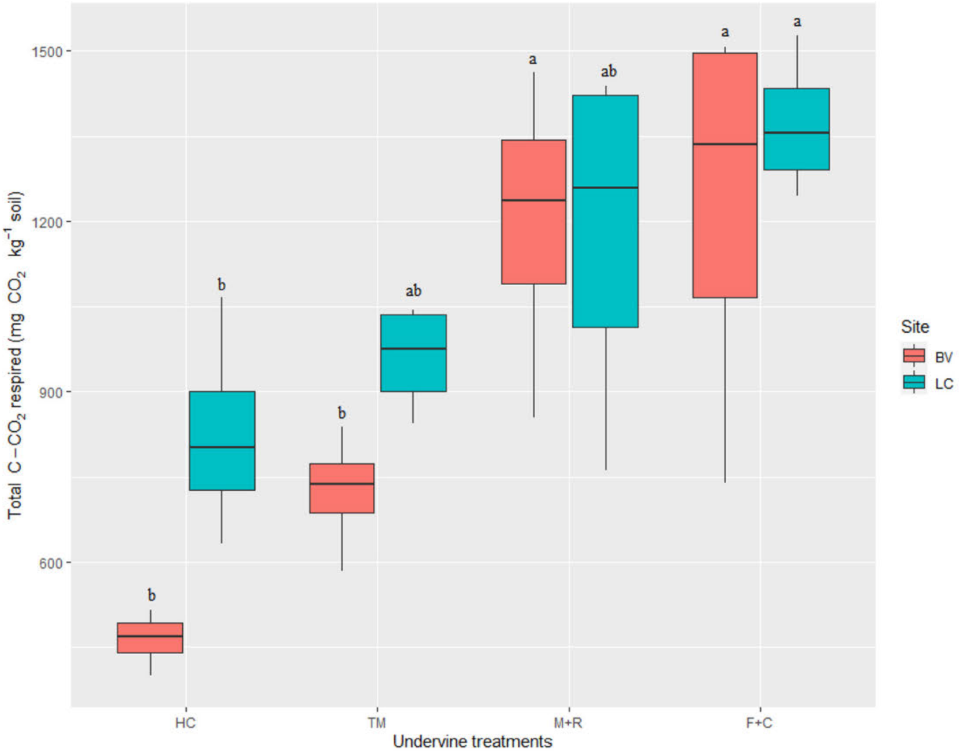

Fig. 3. Boxplots showing median values for total C respired (mg CO_2 kg soil^{-1}) at depth 0–10 cm in soil sampled from beneath under-vine treatments of herbicide control (HC), Triticale mulch (TM), medic-rye (M + R) and fescue-clover (F + C) at sites LC and BV. Letters above treatments (Tukey post-hoc testing) denote significantly different treatments ($p < 0.01$), while those treatments without letters are not significant ($p > 0.05$).

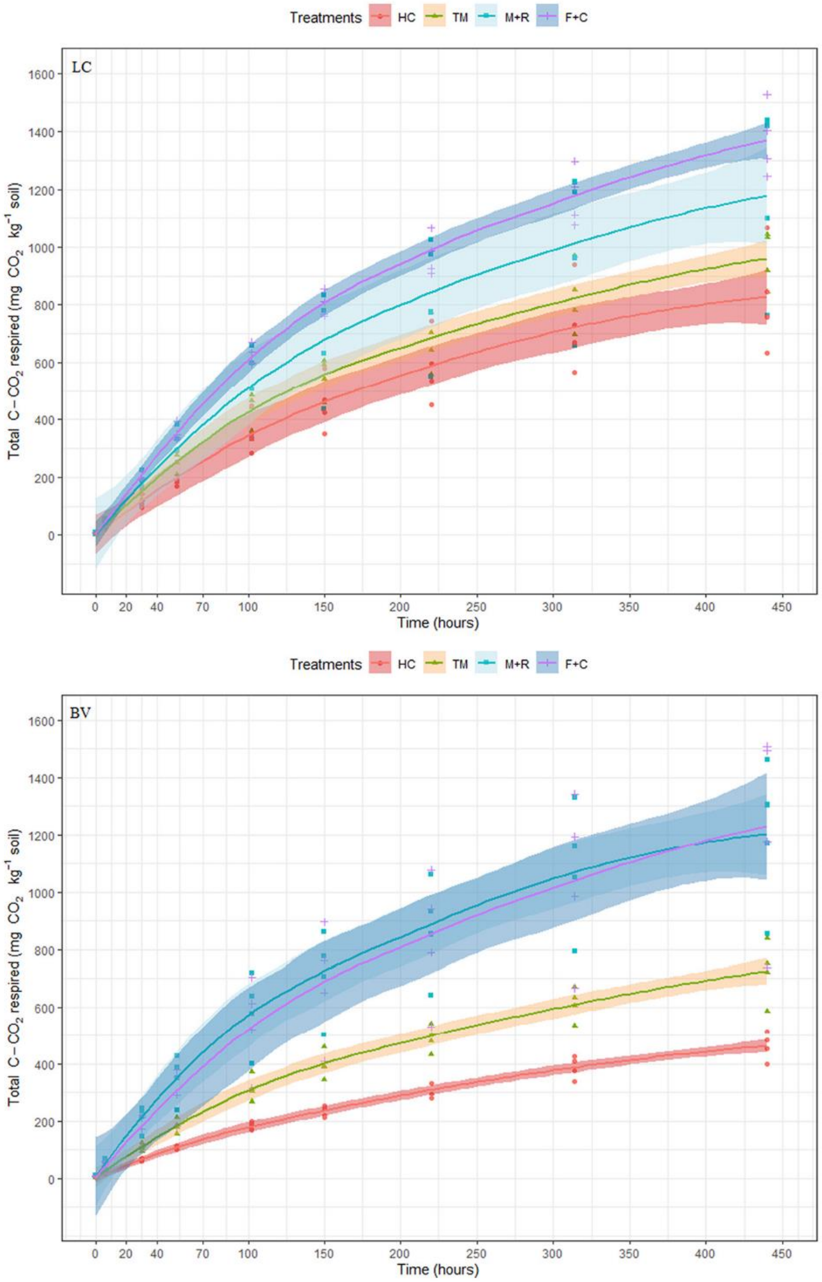

Fig. 4. Scatterplots of modelled CO$_2$ respiration data sampled at several time points (max = 440 h) and listed by site (LC top and BV bottom). Treatments are: HC (herbicide control), TM (Triticale mulch), M + R (medic + ryegrass) and F + C (fescue + clover). Coloured shading around lines represents 95% confidence intervals.

5. Conclusion

Both SOC stocks and labile organic C were increased in the presence of cover crops versus the traditional, herbicide-managed practice. This finding was evident in results for both field sites studied here, despite differences in location, management and a range of intrinsic and extrinsic factors. Taken together, it is clear that cover crops are an effective means of increasing SOC in these vineyards, and on relatively short time scales. In addition, under-vine cover crops increase microbial activity which has additional ecosystem benefits, while also serving as a proxy to validate observed differences in SOC and DOC stocks. Inherent differences (intrinsic and management) likely influenced results, and this demonstrates the importance of specificity when selecting the correct management practice to suit a particular vineyard site and desired outcome. Cover crop combinations that involve a nitrogen-fixing (leguminous) cover crop have been shown to improve soil health and fertility. However, it is essential to select the correct combination of crops to suit the climate and vineyard management strategy. Overly vigorous grasses, such as fescue are unlikely to provide adequate water balance in hot, dry climates, which may be better suited to ryegrass. Future studies pertaining to under-vine (or mid-row) SOC quantification may consider physical and chemical fractionation of soils to further understandings of SOC dynamics under different vineyard management practices.

CRediT authorship contribution statement

Joseph Marks: conceptualisation, data retrieval, data analysis, knowledge, drafting & editing. **Thomas Lines**: conceptualisation, data retrieval, data analysis, knowledge, drafting & editing. **Chris Penfold**: conceptualisation, data retrieval, knowledge, drafting & editing. **Timothy Cavagnaro**: conceptualisation, data retrieval, data analysis, knowledge, drafting & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Part B:

Cover Crops, AMF Inoculum and Decomposition Dynamics Under-Vine

Chapter Four: Under-Vine Cover Crops, AMF and Decomposition Under-Vine

4 A literature review of under-vine cover crops, AMF inoculum and decomposition dynamics

4.1 Introduction

As mentioned in the thesis introduction and chapter structure, the overall nature of this study is best described as two sides of the same coin. That is, both parts pertain to the ecological intensification of vineyards, with an emphasis on under-vine ecology. Ecological intensification, in this sense, refers to the implementation of a functional ecosystem – using cover crops as an agroecosystem service as an alternative to herbicides. Part A of this thesis began with a detailed synthesis of literature on cover crops and soil organic carbon in the vineyard. Part B will begin similarly, with a brief literature review on the ecological themes presented in chapters 5 and 6, on the ecological intensification of the under-vine area by cover crops. In contrast to Part A, this section will focus on ecological theories pertaining to mutualism (cover crops and AMF) and the various mechanisms of under-vine cover crop decomposition. The following is a brief literature review aimed at introducing Part B of the thesis and is not intended for publication beyond this body of work.

4.1.1 *A tradition of cover crops in vineyards*

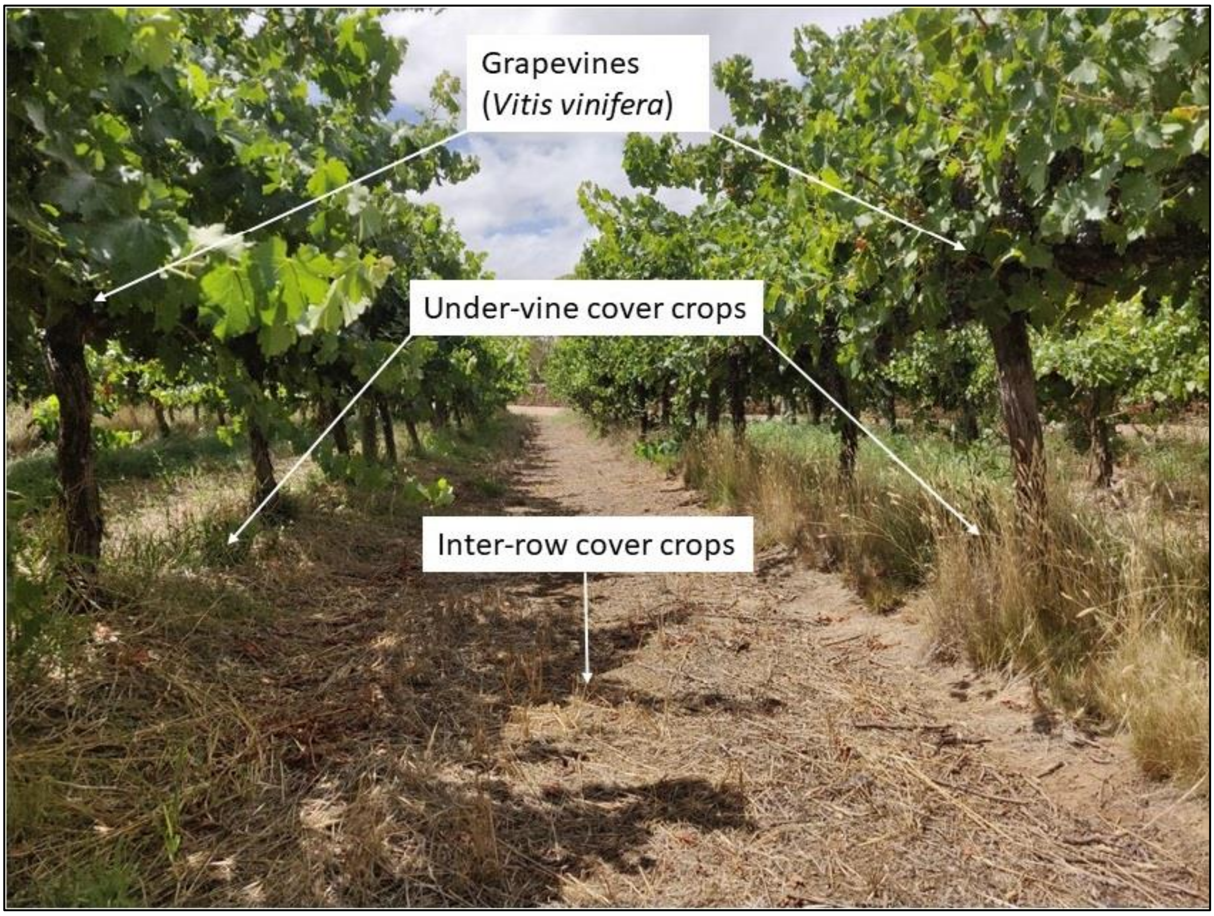

Figure 1. Photograph of vineyard understory showing grapevine row with under-vine cover crops and inter-row cover crops / bare soil (photograph care of Dr. Thomas Lines).

Cover crops have long been used in agriculture systems as a means of fixing nitrogen and stabilising topsoil during crop rotation (Justes 2017). In vineyard systems, cover crops are often sown between rows of vines (inter-row cropping) (Campos *et al.* 2019), with mounting evidence showing multifaceted benefits (Delpuech & Metay 2018).

Vineyard cover-cropping has been shown to affect vineyard and grapevine variables such as: nutrient input and mobilisation (Guerra & Steenwerth 2012; Klodd *et al.* 2016; Pérez Alvarez *et al.* 2015); water retention; soil organic matter; soil stabilisation (Klodd *et al.* 2016; Monteiro & Lopes 2007); weed management (Campos *et al.* 2019); control of vine vegetative growth; (Giese *et al.* 2014; Wheeler *et al.* 2005); increased biodiversity (Guerra & Steenwerth 2012); decreased ground temperature (Collins & Penfold 2014) and; increased arbuscular mycorrhizal associations (see below) with grapevines (Holland *et al.* 2014; Trouvelot *et al.* 2015; Vukicevich *et al.* 2019a).

Biotic and abiotic variables such as grape varietal, vineyard site and climatic nuance (predominantly rainfall and humidity) are essential variables in determining the viability of vineyard management through cover-cropping. The competitive nature of cover crops regarding water usage can prove limiting for vineyards in hot, dry (Mediterranean-type climates) (Celette *et al.* 2009). In contrast, vineyards situated in regions of excessive rainfall and humidity have been shown to benefit from thirsty cover crops through their removal of excess soil moisture (Karl *et al.* 2016).

4.1.2 *Competition and niche overlap*

Competition and niche overlap are two prominent themes in ecology. Competition, in this sense, occurs when the intrinsic niche(s) of an organism overlaps with that of another organism, thus resulting in competition for the limiting resource. Niche overlap – and subsequent competition – lies on a spectrum of intensity, with increased competition occurring when niches overlap to a greater extent. In the context of grapevines and cover crops, niche overlap and competition may occur in response to several resource affinities – not least, water.

Competition for water

Perhaps the greatest concern among viticulturists operating in warm, dry climates is that pertaining to plant-available water and thus the potential for competition between grapevine and cover crop (Costello 2010; Delpuech & Metay 2018). The vast majority of research in vineyards has been conducted on *inter-row* cover cropping, as opposed to *under-vine* cover-cropping (figure 1) (Delpuech & Metay 2018; Morlat & Jacquet 2003). Several experiments conducted on warm, dry vineyards (California and France respectively) with treatments of bare soil vs inter-row cover crops showed little difference in soil water content and vine transpiration (Celette *et al.* 2005; Costello 2010). Experimental evidence from a vineyard planted with (tall fescue/California native grass blend) vs bare soil showed no significant difference in water potential in vine leaves (Celette *et al.* 2005; Ingels *et al.* 2005). Soil depth appears to play a greater role in resource competition (Costello 2010; Klodd *et al.* 2016; Monteiro & Lopes 2007).

Although cover crop roots and grapevine roots may directly compete for water in the upper soil profile, it also worth noting the positive influences of cover crops to enhancing grapevine water availability. Where on one side of the balance exists competition for water, on the other side we can note studies showing that cover crops have the ability to increase infiltration rates by two-fold (Ruiz-Colmenero *et al.* 2013), reduce surface evaporation and enhance plant available water holding capacity (Marks *et al.* 2022; Ruiz-Colmenero *et al.* 2013).

Competition for nutrients

The majority of N mineralisation occurs in the upper soil profile (0-10 cm), increasing competition between shallow-rooted cover crops and grapevines (Peregrina *et al.* 2012; Ripoche *et al.* 2011). In periods of reduced rainfall (inter-seasonal and inter-annual), net mineralisation of N can become reduced, increasing competition (Celette *et al.* 2009). Moreover, the authors found that intercropped grasses competed strongly for inorganic N, owing to their early immergence during autumn when grapevines typically take up N and store it in woody reserves (Celette *et al.* 2009). In addition, the authors noted that where soil was particularly depleted of mineral N, grapevine roots were found to explore under-vine areas where cover crop roots were less present (Celette *et al.* 2009; Klodd *et al.* 2016).

Klodd *et al*. (2016) measured pruning weight, yield and P-concentrations in grapevine petioles from both a control treatment (bare soil) and a treatment with perennial grass (*Festuca rubra*) planted under-vine. Their results showed a marked decrease in P concentration within the cover crop treatment petiole vs the control grapevine (Klodd *et al.* 2016). In contrast to petiole P concentrations, soil P showed no significant difference between cover crop and bare soil treatment and was only affected by soil depth (greater P in 10 cm soil depth than 30 or 60 for both treatments) (Klodd *et al.* 2016). In a similar study, P reduction in grapevines was statistically significant; however, not detrimental to grapevine vegetative growth (belowground and above) (Klodd *et al.* 2016; Pou *et al.* 2011). Moreover, the authors found that cover-cropped grapevine roots explored deeper soil than those grapevines without cover crops (p = 0.054) (Klodd *et al.* 2016). This finding again demonstrates that a niche overlap existed within the aforementioned trial, with both cover crop and grapevine competing for P. In this, grapevine was able to adapt both root width (finer roots to explore the soil matrix) and root length in order to move beyond the niche overlap and explore soil beyond the cover crop root system (Celette *et al.* 2009; Klodd *et al.* 2016).

4.2 Under-vine cover crops and arbuscular mycorrhizal fungi

This section will detail key findings from literature on the role of under-vine cover crops in vineyards and their ability to increase the inoculum potential of AMF. These findings will form the background for Chapter 5 and the research paper titled: *Arbuscular mycorrhizal inoculum potential is unaffected by under-vine cover crops, straw mulch or herbicide management.*

4.2.1 *AMF inoculum potential and under-vine cover crops*

Vitis vinifera is one such species of plant that both supports and benefits from AM colonisation, in part, due to its relatively low root-density and lack of root hairs (Holland *et al.* 2014; Radić *et al.* 2012; Valentine *et al.* 2006). Moreover, there is significant evidence for AM playing a key role in grapevine nutrient uptake (Schreiner & Mihara 2009) and increased soil-water availability (Valentine *et al.* 2006), especially in nutrient-depleted soils. Moreover, AM have been shown to mitigate certain abiotic stresses in grapevines, including increasing nutrient uptake in saline soil (Khalil 2013) and suppressing heavy metal toxicity by increasing binding pathways (Christie *et al.* 2004).

Given the importance of AMF to grapevines and vineyard soil health it will be beneficial to understand whether certain species of cover crop encourage a greater concentration of AMF and AM propagules under-vine or indeed a more rapid colonisation phase. This could be hypothesised through a number of colonisation pathways: spores, root fragments or fungal hyphae (from which terminal division and growth can occur). Radic *et al.* (2012) showed an increased AM-colonisation of grapevines and sporulation of soil. Their research focused on weed crops, *Tanacetum cinerariifolium* and *Plantago lanceolata* and their experimental design used laboratory controlled pots for testing (Radić *et al.* 2012). Pot-grown experiments mimicked the notion of under-vine cover-cropping and showed a significant increase in AM-colonisation (Radić *et al.* 2012). By contrast, Baumgartner *et al*. (2010), tested rye and triticale mulch as cover crops and found there to be no significant difference in AM-colonisation of grapevines between cover crops and the weed-managed control. While their experimental design (field as opposed to laboratory) differed to Radic *et al.*, the most notable difference were species of cover crop (Baumgartner *et al.* 2010). A third study took soils from cover-cropped vineyards and planted young and old grapevines to test whether there was a difference in fungal composition or structure between vine roots (Vukicevich *et al*. 2019b). The authors reported results showing a decrease in arbuscules with older vines, demonstrating a structural shift rather than compositional (Vukicevich *et al.* 2019b).

4.3 Decomposing cover crops under-vine

This section will detail key findings from literature on the ecology of cover crop decomposition under-vine. Specifically, this section will focus on competing mechanisms behind decomposition: the *home-field advantage hypothesis* (HFA) and *substrate matrix interaction hypothesis* (SMI). These findings will form the background for Chapter 4 and the research paper titled: *Home or away: decomposition of under-vine cover crops is unaffected by home-field advantage.*

4.3.1 *The* home-field advantage hypothesis *(HFA)*

Decomposition of plant biomass is an essential ecosystem service and primary pathway for nutrient cycling, carbon turnover and the redistribution of nutrients (Chassain *et al.* 2021). Understanding decomposition dynamics in natural systems versus agroecosystems may present inherent differences, owing to the frequency of disturbances and the heterogeneity of each system (Gießelmann *et al.* 2011). There are several competing theories thought to govern the ecology of decomposition. The *home-field advantage hypothesis* (HFA, hereafter) suggests that plant residues will decompose faster and/or more completely within their *"home"* soil than in an *"away"* soil (that being a soil substrate previously uninhabited by said plant) (Austin *et al.* 2014). The hypothesis posits that preferential endemism can exist between soil microbial communities and their specific plant neighbours (Ayres *et al.* 2009). Over time, these microbial communities become specifically-adapted to decomposing and metabolising compounds that are highly specific to certain plant species or communities (Perez *et al.* 2013). Several studies have employed methods to understand whether HFA exists within particular systems, with contrasting results. Studies involving HFA and decomposition typically opt for a field-trial approach – taking reciprocal litter types and transplanting (or burying) them in litter bags underneath both their own (*home*) soil and also (*away*) soil (Austin *et al.* 2014; Barel *et al.* 2019). A broad study undertaken within several temperate and tropical forests found that HFA had a positive effect on litter decomposition, with litter decomposing 10% faster in 34% of reciprocal transplants. Another study in a tropical rainforest in Brazil employed litter bags to test whether HFA had an effect on decomposition (Ayres *et al.* 2009). Transplanting reciprocal litters beneath contrasting plant communities, the authors found that there were no differences in decomposition and concluded that HFA did not affect decomposition (Ayres *et al.* 2009). Such results may suggest that certain ecosystems have a functional redundancy or resilience within their decomposer communities (Ayres *et al.* 2009). Moreover, it is likely that HFA is more pronounced in distinctly homogeneous systems, where microbial-adaptation to litter type is highly specified, rather than generalist in nature (Gießelmann *et al.* 2011). Additionally, HFA is said to be most pronounced in systems where litter type is composed of such compounds that require specialist decomposers or decomposer communities to metabolise – namely, recalcitrant or resistant litters, such as lignin (Milcu & Manning 2011; Wallenstein *et al.* 2013).

4.3.2 *The* substrate quality-matrix interaction hypothesis *(SMI)*

The *substrate quality-matrix interaction hypothesis* (SMI, hereafter) is, by all accounts, very similar in concept to HFA (Austin *et al.* 2014). The key difference between the two theories is that where HFA proposes almost species-specific decomposer affinity, SMI operates on a more generalist spectrum (Freschet *et al.* 2012; Veen *et al.* 2015). That is, SMI suggests that litter type (or quality) is the main driving force behind decomposition (rate and totality), more so than species-specific interactions (Veen *et al.* 2015). Decomposer communities at the micro-site level may become enzymatically-adapted to breaking down a certain litter type (e.g. one of low C:N ratio); and therefore, under SMI, those same decomposers would more readily metabolise a litter of similar physicochemical composition, rather than one of lower quality (high C:N ratio) (Freschet *et al.* 2012; Veen *et al.* 2015). Where HFA is perhaps more applicable to homogeneous, undisturbed systems, SMI may be more applicable to more frequently disturbed systems, such as vineyards (human and machine traffic). In these agroecosystems, decomposer communities are less highly-specified as they are exposed to numerous (and different) litter types, thus dampening the effects of HFA.

In a meta-analysis of 800 studies, Veen *et al.* (2015) found stronger HFA effects as the dissimilarity between transplanted litter type and host plant community (host litter type) increased. Such results, as the authors suggest, mean that HFA effects are context-dependent and therefore most applicable where substrate quality and litter quality are most dissimilar (Freschet *et al.* 2012; Veen *et al.* 2015). As previously mentioned, the theories of HFA and SMI are two sides of the same coin – each of which are context-dependent. In practical terms; however, HFA appears more applicable to highly endemic systems – where litter type is in or of an *extreme* nature (i.e. highly lignified litter under low water availability) and decomposer communities are highly specified in turn. In the context of vineyard ecology and cover crop decomposition, we might posit that HFA effects would become dampened in a system where disturbance is frequent and litter type heterogeneous. Such research remains relatively unexplored, thus providing ample context for in-field and laboratory trials to understand whether endemism and specified decomposer communities exist under-vine between different management practices.

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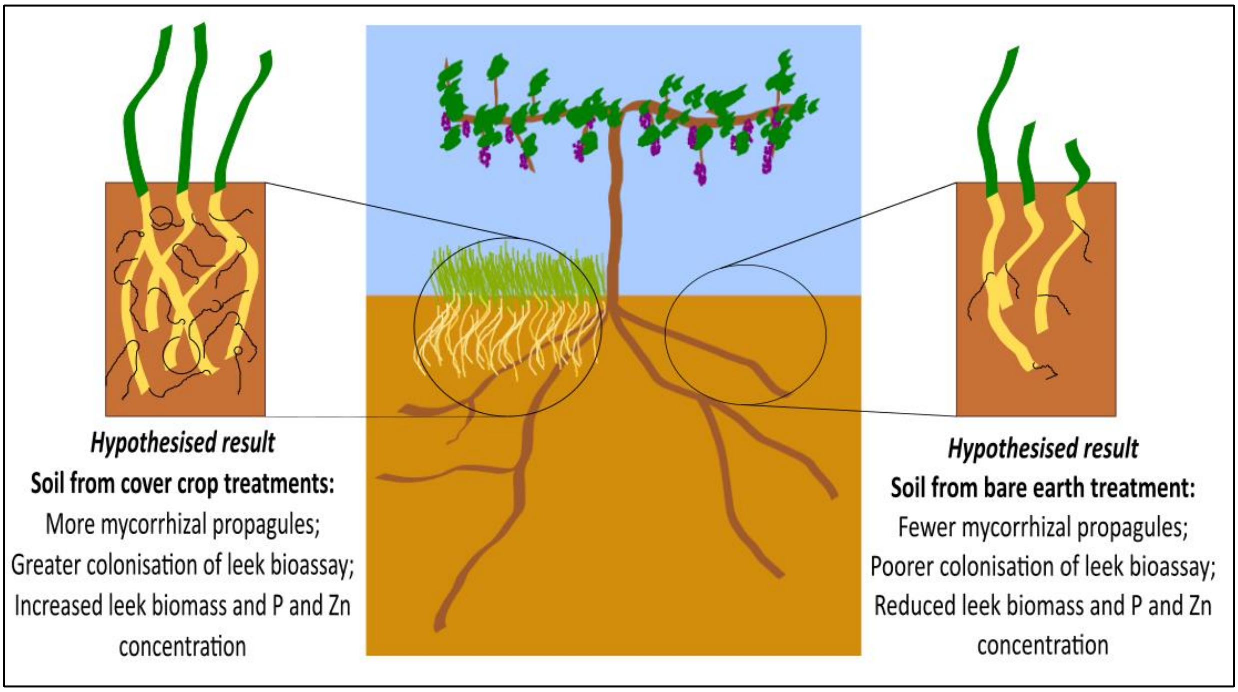
Chapter Five: Under-Vine Cover Crops and AMF Inoculum.

5 Arbuscular mycorrhizal inoculum potential is unaffected by under-vine cover crops, straw mulch or herbicide management

These were my first experiments and first scientific manuscript.
As a first draft it came back with a lot of red text… I was humbled.
Since then, each manuscript draft has been returned with less and less red text –
I guess I've learnt a thing or two…



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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Abstract

Purpose: Several studies have sought to quantify the influence of vineyard floor management on arbuscular mycorrhizal (AM) inoculum potential, however, few have investigated the effect of under-vine cover crops. To investigate this, seven treatments (five cover crops, one surface mulch and one herbicide-managed control) were established under-vine in a vineyard site at Langhorne Creek, South Australia in 2014. Following a four-year interval (2014-2018) and then a six-year interval (2014-2020) soils were sampled under-vine and two glasshouse trials were undertaken. The glasshouse trials aimed to test the hypothesis that cover crops planted directly under-vine increase the AM inoculum potential of leek (sp. *Allium porrum*) trap plants (Exp. 1) and medic (*Medicago truncatula*) and ryegrass (*Lolium rigidum*) (Exp. 2) when potted in soil sampled from under-vine treatments.

Method: Arbuscular mycorrhizal fungi are known to increase plant nutrient assimilation and therefore enhance growth in many terrestrial plants, after an eight-week growth period, leek plants were harvested and analysed for AM colonisation and root/shoot biomass to understand if a relationship could be established between the presence of cover crops and increased AM inoculum potential. Furthermore, prior to the second pot-growth experiment, a spore count was conducted on soil samples to test for differences in spore abundance among treatments.

Results: While some significant differences were observed in mean plant biomass, there were no significant differences in leek, medic or ryegrass colonisation by AMF. Additionally, there were no significant differences in the number of AM spores between treatments.

Conclusion: Results suggest that sources of AMF inoculum (spores mainly) remain present and viable in cover crop and bare earth treatments. This may be due to the encroachment of mid-row vegetation and perennial grapevines offering buffering to inoculum.

Keywords:

Under-vine; cover crops; mulch, herbicide; inoculum potential; mycorrhiza.

5.1 Introduction

Sustainable viticulture is an increasingly-important practice and one that encompasses multiple ecosystem processes and vineyard floor management practices. Sustainability in viticulture largely reflects a reversion to a more naturally-managed agroecosystem: decreasing the reliance on herbicide, pesticide and fertiliser inputs, while encouraging macro and micro biodiversity both above and below-ground (Celette *et al.* 2005; Guerra & Steenwerth 2012). A steady adoption of sustainable viticultural practice has been driven, in part, by observable and quantifiable land degradation including but not limited to: soil erosion, herbicide-resistance and a decline in biodiversity (Guerra & Steenwerth 2012; Wheeler & Crisp 2011). Cover-cropping is one such management practice aimed at enhancing soil-grapevine (sp. *Vitis vinifera*) interactions and the ecological intensification of the vineyard, while at the same time reducing the need for chemical inputs and invasive farming practices (Celette *et al.* 2005; Guerra & Steenwerth 2012; Peregrina *et al.* 2012).

The use of cover crops in vineyards has a long-standing tradition, especially when sown in the mid-row. Anecdotal evidence has given way to mounting statistical data, showing positive influences of cover crops (species-specific) on vineyard soil and grapevine health. Such evidence has shown cover crops to influence nutrient cycling; water relations; pathogen-resistance; biodiversity; erosion-prevention; organic carbon content and; diversity of arbuscular mycorrhizal fungi (AMF), among other variables (Celette *et al.* 2005; Guerra & Steenwerth 2012; Monteiro & Lopes 2007). Although the weight of evidence has steadily increased for mid-row cover crop management, less experimental data has been accrued on *under-vine* management practice (Jordan *et al.* 2016). Sowing cover crops directly beneath grapevines may increase the effect of the ecological interaction both below-ground (roots) and above-ground (canopies), potentially exacerbating both positive and negative effects (Karl *et al.* 2016). One such targeted interaction that may be affected is the inoculum potential of AMF due to an increase in root biomass owing to the presence of cover crops.

Arbuscular mycorrhizal fungi are ubiquitous symbionts of approximately 80% of terrestrial plant species (Smith & Read 2008). The relationship is one of colonisation and exchange as arbuscular mycorrhizal (AM) hyphal networks infiltrate plant root cells, taking up plant-assimilated carbon in exchange for highly immobile nutrients such as phosphorus and zinc (Cavagnaro 2008; Smith & Read 2008). Grapevine (sp. *V. vinifera*) benefit from AM colonisation owing to their thicker root system and relative lack of fine root hairs (Holland *et al.* 2014; Radić *et al.* 2012). Moreover, soil health has been shown to benefit from increased hyphal networks through nutrient cycling; organic carbon content; soil structure and; water infiltration (Schreiner & Mihara 2009; Valentine *et al.* 2006). It is well-established that management practices, including soil cultivation and the inclusion of cover crops, can impact the formation of AM (Bowles *et al.* 2017). Several studies have focused on the relationship between cover crop presence and increased AM diversity, yet few have sought to quantify inoculum potential, especially under-vine (Baumgartner *et al.* 2010; Vukicevich *et al.* 2019a).

The primary aim of this study was to determine the influence of five different species / combinations of cover crop, one straw mulch and one herbicide (bare earth) control on the inoculum potential of AMF under-vine. This aim was addressed in two separate experiments, using soil collected from a long-term field trial that has been established to study impacts of under-vine management options. In the first experiment, leek plants (sp. *Allium porrum*) were used as trap plants to test the hypothesis that leeks grown in soils sampled from under-vine cover crops would be significantly more colonised by AMF than those grown in non-cover crop soils (increased inoculum potential). To further understand inoculum potential between treatments, a second pot-growth experiment was undertaken, using medic (*Medicago truncatula*) and ryegrass (*Lolium rigidum*). In both experiments, the aims and hypotheses remained the same. Furthermore, it is hypothesised that if the inoculum potential (and therefore root colonisation) is significantly different between treatments then this will also present in plant biomass (correlating with root colonisation).

5.2 Materials & Methods

5.2.1 *The trial site*

Available to this project was a long-term field trial which was comprised of seven under-vine treatments (five cover crop combinations, a surface mulch and bare-earth control); see below. The under-vine trial was located in a Cabernet Sauvignon block, located at a site in the Langhorne Creek wine region (-35.302”S, 138.952”E), Australia. The soil is a Brown Sodosol to a depth of approximately one metre (Hall *et al.* 2009). The vineyard was established in 2014 with 1.8 m vine spacing. The vines were on their own rootstock (Cab LC-10), trained a single cordon trellis, and saw box pruned to 15-18 t ha^{-1}. The site was irrigated at a rate of approximately 3.5 ML ha^{-1} and dripper spacing of 0.6 m. The mid-row, which was not irrigated, was vegetated with Triticale straw and managed using a combination of slashing and heavy rolling during the growing season. The under-vine region of the vineyard floor – the subject of this study – was traditionally managed at the site by using herbicides to control volunteer weeds in a 300 mm wide strip beneath the vines. In 2014 an under-vine management trial was established at the site, from which soils were collected for this study. The trial was comprised of seven treatments, including five cover crop treatments sown under-vine using a towed mechanical no-till disc seeder; cereal straw mulch treatment applied at a rate of 50 t ha $^{-1}$; and a ‘business-as-usual’ herbicide control treatment which involved the use of a combination of glyphosate (during vine dormancy) and glufosinate ammonium (when vines were active), applied thrice yearly. Full details of the under-vine treatments are presented in Table 1.

The under-vine field trial, from which soils were collected for the experiments presented here (see below), was conducted using a randomised complete block design (RCBD), which each of the seven treatments replicated four times (n = 28). Each experimental plot, of which there were 28 (i.e. seven treatments × four replicates/blocks), was comprised of nine vines (i.e. three panels). The mid-row was uniformly managed across the site, with minimal intervention.

5.2.2 *Sampling and processing*

The cover crops included in this study follow an annual growth cycle, flourishing in cooler, wetter months (May – September), while declining in warmer months to coincide with reduced recourse competition with grapevines during their flowering and fruiting season (October – January/February). Thus, soil sampling was conducted in October 2018 (experiment 1) and October 2020 (experiment 2) (austral spring; grapevine bud burst) where it was anticipated that the inoculum potential of AMF would be near its peak. From each experimental plot, three soil samples were collected from the 0-10 cm soil layer using a stainless steel core (10 cm diameter) and slide hammer. These three samples were then pooled and mixed at the individual plot level. Thus, there were four replicate soils samples for each of the seven experimental treatments (i.e. is sample per replicate/block), giving 28 soil samples in total. Immediately following collection, soil samples were returned to the laboratory, air-dried (40°C in an oven for 72 hours), and then sieved to <2 mm. Within the laboratory all soil samples were divided into three subsamples, except for the 2020 soil samples, which were divided into four. The first subsample was sent to the Australian Precision Agricultural Laboratory (APAL) in Hindmarsh, South Australia (https://www.apal.com.au, last accessed May 2020) for analysis of soil physicochemical properties: Plant-available (Colwell) P, total N and Zn. The second subsample was used to determine water-holding capacity (WHC) of the different soils, as described previously (Cavagnaro 2016). The third and fourth subsamples were used in experiments 1 and 2, as follows.

5.2.3 *Spore count*

Three subsamples of each soil replicate (n = 28) were collected and wet-sieved between a 53 and 250 μm sieve (Gerdemann & Nicolson 1963). Each subsample weighed 3.33 g, totalling 10 g per soil replicate. The content of the 53 μm sieve was collected and washed using a sugar-gradient centrifugation (Brundrett *et al.* 1996). After the second centrifugation, the spores were again collected on a 53 μm sieve and sugar residues washed off using tap water. Following, the spores were transferred to a nematode counting dish. The absolute number of AMF spores per dish were counted under a dissection microscope at 80-120× magnification.

5.2.4 *Exps. 1 and 2: glasshouse bioassay and harvest*

The AMF inoculum potential of the soils was assessed in two separate experiments. In the first Experiment (Exp. 1 hereafter) seedlings of leek (*A. porrum* L.) were grown in a glasshouse bioassay in October 2019. Leek plants were selected as the bioassay test plant species for Experiment 1, owing to its high affinity for forming AM (Torelli *et al.* 2000), and not being present in the plant communities at the field sites (negating potential home-field advantage). In the second, Experiment 2: seedlings of medic (*M. truncatula*) and ryegrass (*L. rigidum*) were grown (separately) in a glasshouse bioassay in October 2020.

Table 1. List of seven experimental treatments with common name, species name and biological / ecological properties selected for experimental / industry-relevant significance. For the purpose of clarity, treatment names will henceforth be referred by their abbreviated code names, i.e. HC = Herbicide control; TM = Triticale mulch; M+R = Medic + ryegrass; M+M = Medic + medic (cultivar combination); KC = Kasbah Cocksfoot; F+C = Predator fescue + Strawberry clover and; WG = Wallaby grass.

Treatment code	Common name	Species name	Properties
HC	Control (herbicide)	N/A	Traditional management practice, vegetation cover suppressed
TM	Triticale mulch	N/A	Soil stabilisation; temperature regulation and; soil moisture enhancement (Prosdocimi *et al.* 2016; Thomson & Hoffmann 2007)
M+R	Safeguard ryegrass/ Scimitar	*Lolium rigidum* / *Medicago polymorpha*	Annual grasses; fibrous roots for soil stabilisation; water infiltration potential; high N-uptake therefore grown in combination with medic (N-fixer)
M+M	Angel strand / Sultan barrel medic	*Medicago littoralis* / *Medicago truncatula*	N-fixers with low C:N ratio and useful for experimentation (Kumar & Goh 2003)
KC	Kasbah cocksfoot	*Dactylis glomerata*	Summer dormancy (can be suppressed if irrigated) and weed-suppression (ideal for dry climates) (Weston 1996)
F+C	Predator fescue / Strawberry clover	*Festuca* sp. / *Trifolium fragiferum*	Fescues can be overly vigorous – an advantage in wetter climates – and grown best in combination (Celette *et al.* 2005)
WG	Wallaby grass	*Rytidosperma caespitosa*	Native grass with adaptive traits to water-reduction; weed-suppression and; increased native microbial biodiversity (Mitchell 2003)

Table 2. Physicochemical properties of soils collected from beneath treatments of cover crops at the Langhorne Creek vineyard. N.B the experimental treatments at the site are described in Table 1. Values are Mean ± S.E.; n = 32 (see Table 1 for all treatment codes).

2018 SOIL NUTRIENT DATA	**HC soil**		**TM soil**		**M+R soil**		**M+M soil**		**KC soil**		**F+C soil**		**WG soil**	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Total N (%)	0.17	0.01	0.17	0.01	0.25	0.04	0.19	0.01	0.21	0.02	0.21	0.01	0.19	0.00
Plant-available (Colwell) P (mg kg^{-1})	19.5	4.03	43.2	8.51	22.5	4.2	20.0	2.86	50.8	13.8	29.3	7.2	27.3	6.3
Zn (mg kg^{-1})	4.2	0.6	4.2	0.62	5.2	0.9	4.6	0.8	4.8	0.5	3.8	0.8	4.3	0.4
2020 SOIL NUTRIENT DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Total N (%)	0.19	0.01	0.20	0.01	0.22	0.02	0.22	0.01	0.20	0.02	0.21	0.00	0.20	0.01
Plant-available (Colwell) P (mg kg^{-1})	20.25	5.41	44.50	9.02	22.29.25	5.81	22.75	6.41	38.50	5.27	31.50	8.41	57.00	12.60
Zn (mg kg^{-1})	3.95	0.37	3.30	0.91	4.05	0.36	3.23	0.48	3.78	0.70	3.15	0.43	2.75	0.39

The use of medic and ryegrass were later selected (Exp. 2) to account for the possibility that leek affinity (Exp. 1) for AM symbiosis was such that treatment effects may have been masked. Experiments 1 and 2 were performed in the same manner, as follows. To an 800 mL plastic free-draining pots, 500 g of soil was added and packed to a bulk density of 1.3 g cm^{-3} (equivalent to bulk density at the field site). Soil in each pot was then watered to 80% WHC and left for one week to allow soil microbial biomass to re-equilibrate (Schimel *et al.* 2007). To each pot four pre-germinated seeds of Leek (Experiment 1), or Medic or Rye (Experiment 2) were planted. Prior to planting the seeds were surface sterilised by washing in a 4.125% NaOCl (sodium hypochlorite) solution, followed by rinsing with Reverse Osmosis (RO) water to remove traces of NaOCl and commercial fungicide. The seeds were then placed on moist filter paper in Petri dishes and incubated at 25°C in the dark for three to five days, when approximately 10 mm of the radical had emerged. Following sowing of the seeds, the pots were placed in a glasshouse on the Waite campus of the University of Adelaide and allowed to grow for a period of eight weeks. Plants were watered thrice weekly to 80% of WHC with RO water, and rotated on the glasshouse bench. Two weeks after sowing the number of seedlings was thinned to two per plant pot. Glasshouse daily temperatures ranged from 14°C (min) to 30.1°C (max) and daily recorded relative humidity (RH) ranged from 23.9% RH (min) to 21.1% RH (max).

After an eight-week growth period plants were destructively harvested, as follows. Plants were washed free of the soil in RO water. All fresh tissue (with a subsample of root tissue) was weighed and oven dried at 50°C and root dry weight (RDW) and shoot dry weight (SDW) was collected. A subsample of fresh root tissue was weighed for AMF root-colonisation analysis, using the gridline intersect method (Giovannetti & Mosse 1980). Roots were washed in KOH (10% *W/V*) and stained with a 1:1 solution of 50 mL 5% ink (black Schaeffer) to 50mL white vinegar, prior to microscopic analysis (SZ-STS Olympus microscope) of root-grid intersects (20× magnification).

5.2.5 *Statistical analyses*

Prior to significance testing, each treatment variable was tested for normality and homoscedasticity using the Wilkes-Shapiro test, and, if necessary, the data were transformed using either log, square root or an exponent function to achieve normality. Those variables that violated ANOVA assumptions of normality after transformations were analysed using a non-parametric approach (Friedman Test). Owing to the randomised complete block design (RCBD) of the field trials (and glasshouse trial), the data (for each site separately) was analysed using two-way ANOVA with *Treatment* and *Block* as factors in the analysis. *Block* was included in our analysis to account for any underlying spatial heterogeneity of the vineyard sites. Where two-way ANOVA revealed a significant difference (P <0.05), Tukey HSD *post-hoc* analysis was used to determine pairwise comparisons between significantly different treatment means. As noted above, where the assumptions of normality could not be met, the data were analysed using a non-parametric (Friedman Test) approach analogous to a two-way ANOVA, with further *post-hoc* analysis used if testing showed significance. All statistical analyses were conducted using R open source statistical software (R version 3.6.3) (Team 2013) with additional agricultural data package, “agricolae” and visual output package, “ggplot2”. Alpha was set at 0.05.

5.3 Results

5.3.1 *AM colonisation of leek roots (Exp. 1)*

The aim of experiment 1 was to assess the impact of different cover crop treatments on the potential for AMF to colonise the roots of a highly mycotrophic plant species (Leek) that does not occur on the site from where the soil was collected. The inoculum potential of AMF was high in leeks grown across all soil treatments (Fig. 1), with the roots of all leek plants well-colonised by AMF. Across all treatments, the mean percentage of leek plant roots colonised by AMF were 66 ± 0.6%. There was no significant difference in inoculum potential of the soils from the different treatments, including the herbicide control, as indicated AMF colonisation of the leek plants. There were, however, significant ($p < 0.01$) differences in root length colonised (RLC) between treatments (Table 3). Specifically, RLC was significantly higher in leeks grown in treatments of KC and WG treatments, compared to the combined M+R treatment. This was a reflection of differences in plant growth. Specifically, the leek biomass (root dry weight and shoot

Table 3. Plant biomass and arbuscular mycorrhizal colonisation data for leek, medic and ryegrass plants when grown in treatments of soil sampled under-vine at Langhorne Creek. N.B Significantly different treatment means are denoted by different letters ($\alpha = 0.05$). Values are Mean ± S.E.; n = 32 (see Table 1 for all treatment codes).

PLANT BIOMASS & AMF DATA	**HC soil**		**TM soil**		**M+R soil**		**M+M soil**		**KC soil**		**F+C soil**		**WG soil**	
2018 LEEK DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	69.64	2.20	68.95	4.82	64.42	4.01	65.07	3.75	58.30	8.86	79.19	4.64	73.50	4.05
Root length colonisation (cm)	418.7$_{ab}$	18.1	357.3$_{ab}$	67.8	115.5$_{b}$	37.6	386.1$_{ab}$	62.2	561.4$_{a}$	131.9	311.9$_{ab}$	68.6	492.2$_{a}$	95.9
Shoot total weight (g)	0.36$_{ab}$	0.16	0.38$_{ab}$	0.09	0.16$_{b}$	0.07	0.56$_{ab}$	0.12	0.80$_{a}$	0.16	0.37$_{ab}$	0.07	0.46$_{ab}$	0.06
Root total weight (g)	0.29$_{ab}$	0.10	0.29$_{ab}$	0.08	0.11$_{b}$	0.04	0.28$_{ab}$	0.04	0.55$_{a}$	0.07	0.22$_{b}$	0.04	0.42$_{ab}$	0.09
	HC soil		**TM soil**		**M+R soil**		**M+M soil**		**KC soil**		**F+C soil**		**WG soil**	
2020 MEDIC DATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	83.34	3.96	81.59	2.25	83.72	6.81	85.28	1.81	75.98	8.14	72.53	3.84	69.49	6.37
Root length colonisation (cm)	376.6	64.6	484.8	69.1	397.6	67.9	439.0	68.8	403.3	84.7	353.9	49.0	459.4	85.7
Shoot total weight (g)	0.61	0.16	1.84	0.15	1.03	0.16	1.33	0.22	0.95	0.30	1.14	0.19	1.23	0.38
Root total weight (g)	0.12	0.04	0.17	0.02	0.09	0.03	0.14	0.02	0.12	0.03	0.11	0.01	0.16	0.05
	HC soil		**TM soil**		**M+R soil**		**M+M soil**		**KC soil**		**F+C soil**		**WG soil**	
2020 RYEGRASSDATA	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
AM root colonisation (%)	19.10	6.55	14.25	3.10	40.05	13.60	28.82	9.05	20.03	5.04	11.37	2.44	10.84	1.80
Root length colonisation (cm)	143.5	65.2	186.9	39.9	249.5	102.5	265.9	141.2	230.5	41.9	161.0	68.3	91.3	32.1
Shoot total weight (g)	0.37$_{b}$	0.14	1.09$_{a}$	0.05	0.70$_{ab}$	0.15	0.65$_{ab}$	0.21	0.78$_{ab}$	0.13	1.05$_{ab}$	0.18	0.55$_{ab}$	0.11
Root total weight (g)	0.28	0.13	1.05	0.37	0.35	0.11	0.53	0.20	0.72	0.10	0.80	0.17	0.50	0.25

dry weight) was significantly different between treatments ($p < 0.01$) (Table 3). For both SDW and RDW, leek plants grown in KC soil showed significantly higher total biomass than all other treatments, with those leeks grown in M+R soil presenting the lowest overall biomass. There were no significant differences in leek biomass when grown in HC (control) soil vs any other under-vine treatment. No significant differences in soil nutrients among the treatments were observed (Table 2); mean total N = 0.20 ± 0.01%; total Colwell P = 30.36 ± 3.31 mg kg^{-1} and; total Zn = 4.43 ± 0.24 mg kg^{-1}.

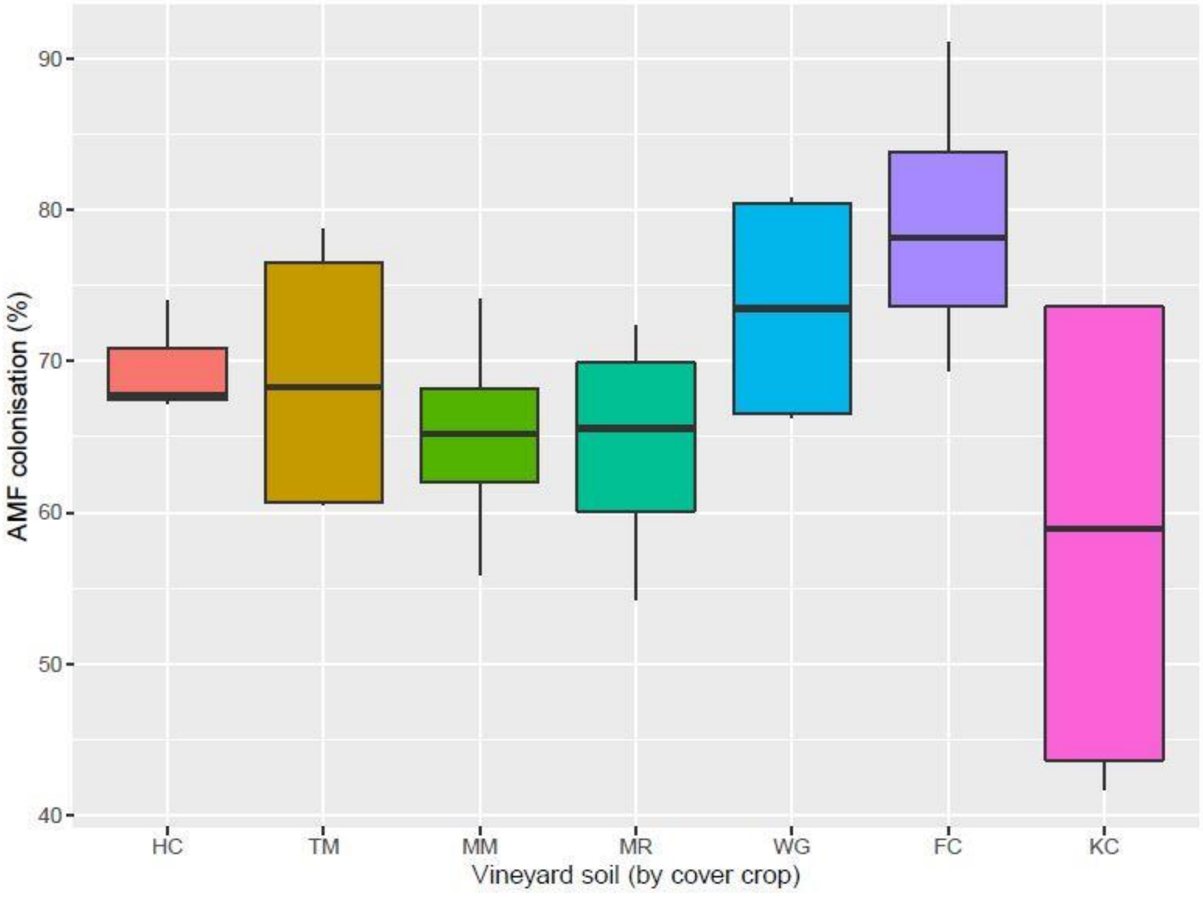

Figure 3. Boxplot showing percentage of leek roots colonised by AMF when grown in soils sampled from under-vine treatments at Langhorne Creek (Exp. 1). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant ($p > 0.05$).

5.3.2 *AM colonisation of medic and ryegrass roots (Exp. 2)*

The aim of experiment 2 was to assess the impact of different cover crop treatments on the potential for AMF to colonise the roots of two of the cover crop species grown on the site from where the soil was collected. Roots of both medic and rye were well colonised by AMF (Fig X). Across all treatments, the mean percentage of medic roots colonised by AMF were 78.9 ± 2.1%, while the mean percentage of ryegrass roots colonised by AMF were 20.6 ± 3.0% respectively ($p > 0.05$). The inoculum potential of AMF differed considerably between medic and ryegrass plants, which is to be expected, given their different affinities for symbiosis with AMF; however, there were no significant differences in AM colonisation of roots between the experimental treatments. Similarly, there were no significant differences in root length colonised (RLC) of medic or ryegrass, between the different soil treatments ($p > 0.05$). Medic plants showed no significant differences in either root or shoot biomass across all treatments ($P>0.05$). Ryegrass plants showed significant differences ($P<0.03$) in total shoot weight, with ryegrass grown in treatments of TM soil presenting the greatest biomass vs those grown in HC soil. Similarly for soil from Exp. 1, no significant differences in soil nutrients among the treatments were observed (Table 2); total N = 0.20 ± 0.05%; total P = 34.82 ± 4.05 (mg kg^{-1}) and; total Zn = 3.46 ± 0.69 (mg kg^{-1}).

Spore count analysis was conducted on 2020 soil samples (Figure 5). Soil sampled from treatment WG showed the highest average spore count (41.75 ± 7.66 spores g^{-1} soil), while soil sampled from TM showed the lowest average spore count (21.2 ± 3.94 spores g^{-1} soil). The overall average spore count across all treatments was 33.47 ± 2.15 spores g^{-1} soil. Regardless of the observable differences in treatment means, we were unable to report significant treatment differences ($P>0.05$).

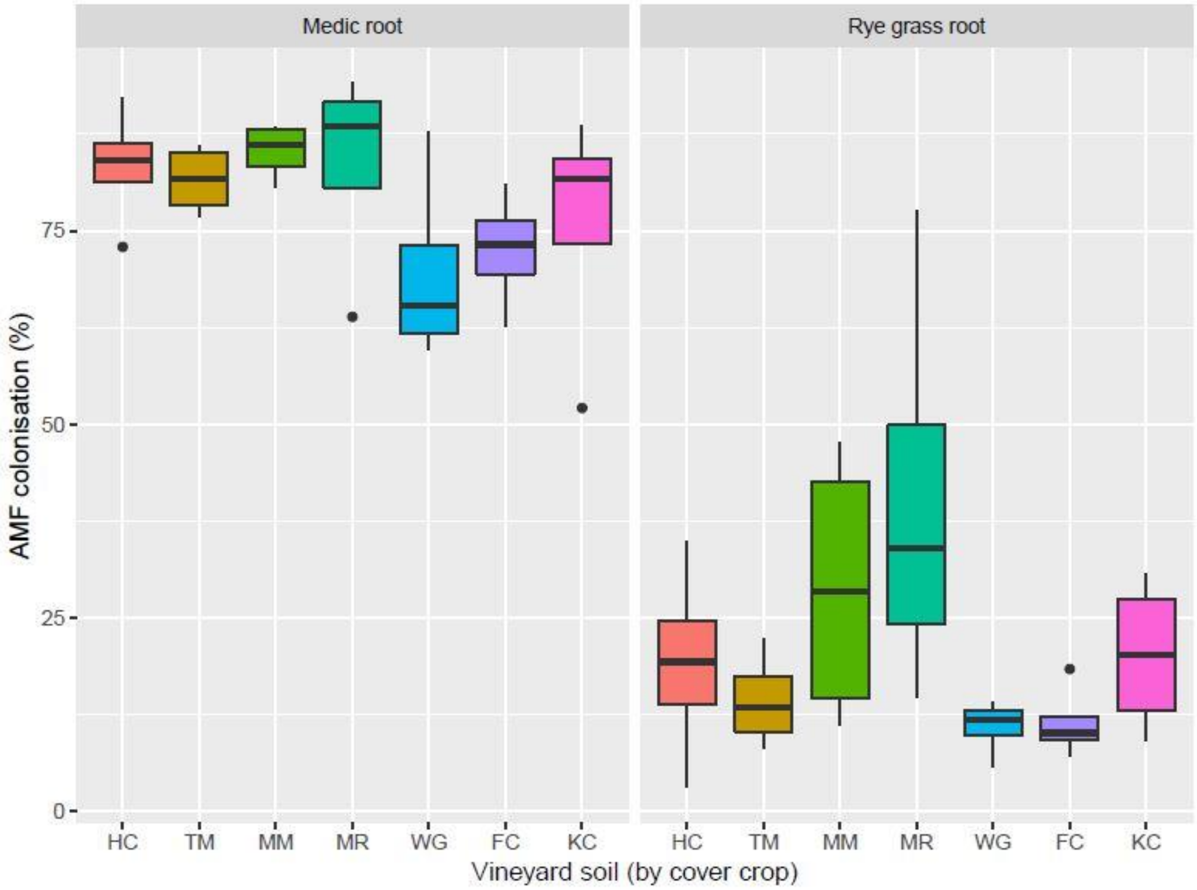

Figure 4. Boxplot showing percentage of medic and ryegrass roots colonised by AMF when grown in soils sampled from under-vine treatments at Langhorne Creek (Exp. 2). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant ($p > 0.05$).

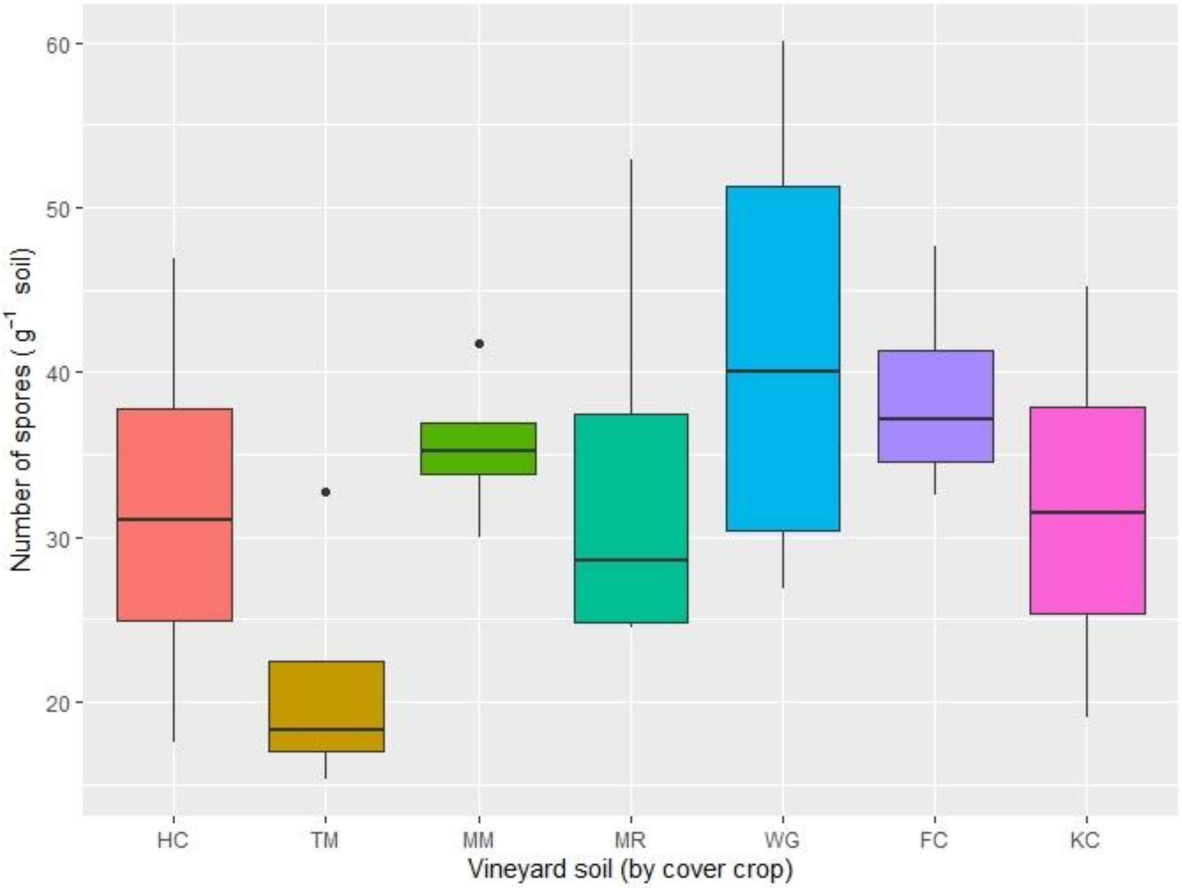

Figure 5. Boxplot showing number of AMF spores per gram of soil in treatments of soil sampled under-vine from Langhorne Creek (Exp. 2). Treatments codes are as follows: HC = herbicide control; TM = Triticale mulch; MM = medic combination; MR = medic/ryegrass; WG = wallaby grass; FC = fescue/clover; and KC = Kasbah Cocksfoot. Boxplot shows median and interquartile range. Treatment means were non-significant ($p > 0.05$).

5.4 Discussion & Conclusion

5.4.1 *Exp. 1: leek trap plants and an initial hypotheses*

After an eight-week growth period, there were no significant differences in the percentage of roots colonised by AMF, which is suggestive of no significant differences in the inoculum potential of the under-vine soil treatments. Leek biomass (root, shoot and, subsequently, root length colonised) was significantly different, however, without evidence for differences in AM colonisation, this is likely a consequence of differences in nutrient contents of soils from the experimental plots, particularly in soil sampled treatments of KC (see Table 2). The initial hypothesis suggested that, after a four-year growth period, under-vine soils inhabited by cover crops would have an increased AMF inoculum potential compared with those managed with herbicide. Initial experimentation used leek (sp. *A. porrum*) as a suitable trap plant to avoid limiting results through possible home-field advantage interference, however in light of the above-mentioned results, two potential issues with initial hypothesis-testing: 1) *A. porrum* is a highly mycotrophic species (Smith & Read 2008) – a factor that could have inhibited treatment-based results owing to inherent plant-phenology and, 2) we did not conduct an initial spore count to test whether this fundamental source of inoculum differed between soil treatments prior to pot-growth experimentation. It became clear that these further questions needed to be explored prior to a definitive conclusion being asserted, and thus, a second experiment was conducted.

5.4.2 *Exp. 2: mycotrophic vs non- mycotrophic trap plants*

To further explore our initial hypothesis, a second line of inquiry was proposed, this time quantifying inoculum potential from the same treatment plots, however now using two different plants: medic and ryegrass (mycotrophic vs non-mycotrophic). This second experiment not only investigated inherent mycotrophic differences in root colonisation (medic vs ryegrass) but also allowed for temporal effects of inoculum deposition to become more pronounced. A spore count was also conducted on these soils, however, there were no significant differences in spore counts, thus answering one of the primary questions raised from Exp. 1. Similar to leek, medic is also highly mycotrophic (Smith & Read 2008), however, ryegrass is far less so and was thus chosen to mitigate any inherent plant phenology that might mask treatment-based differences (as seen in Exp. 1). Colonisation of medic roots was higher than ryegrass roots, owing to the highly mycotrophic nature of medic (Smith & Read 2008). Despite differences in total colonisation (between trap plant species), there were no observable treatment effects (between the same trap plants grown in different soils). Although our initial hypothesis may not apply, the differences in colonisation between trap plants provides insight into management practice. Inoculum potential is only *potential*, with hyphal networks only forming if a mycotrophic plant (and root system) is present. Therefore, we may speculate that under-vine cover crops consisting of highly mycotrophic plants (e.g. medic) have the *potential* to become more highly colonised and thus support a greater abundance (network) of hyphal structures (Bowles *et al.* 2017).

5.4.3 *Mycorrhizal spore inoculum is unaffected by herbicide treatment*

In the sustained absence of under-vine vegetation (i.e. the herbicide and mulch treatments), we hypothesised that the AMF inoculum potential would be lower than where under-vine cover crops were present. This was, however, not the case. Impacts of herbicide management on AMF vary considerably among studies and can, by nature, be both *direct* (deleterious or neutral impact on fungi) or *indirect* (removal of host plants leading to absence of AMF) (Rose *et al.* 2016). Druille *et al.* (2013) report a negative correlation between rate of glyphosate application to soil, and spore viability and subsequent root colonisation. Moreover, they concluded that glyphosate became deleterious to AMF viability and root colonisation only when applied directly to the soil but not when applied to plant foliage (Druille *et al.* 2013). Moreover, while the literature suggests that AM hyphae may be prone to the detrimental effects of herbicides, less is mentioned on spore viability and capacity to buffer against herbicide leachate (Baumgartner *et al.* 2010). Based on results from a number of previous studies, even with twice the rate of conventional field application, several herbicide compounds showed little effect on inoculum potential (Mujica *et al.* 1999; Pasaribu *et al.* 2013). We can therefore conclude that herbicide-management under-vine may not necessarily result in a decrease in fungal inoculum potential. Indeed, these findings concur with our own, where soils from experiment 2 showed no significant differences in the number of spores between treatments. Herbicides may indirectly affect AMF inoculum potential merely due to the absence of host plants – a notable condition in cropping systems known as *long-fallow disorder* whereby the prolonged absence of host plants diminishes the ability of AM propagules to colonise subsequent crops (Thompson 1987). Although a well-regarded symptom of host-absence, *long-fallow disorder* may have been mitigated in our own field trial due to the buffering presence of grapevines and mid-row vegetation. Based on evidence from a meta-analysis conducted by Bowles *et al.* (2017), we might have predicted that maintaining under-vine cover would serve to enhance AM inoculum potential, however, this was not the case. Several studies have concluded similar results, notably that conducted by Baumgartner *et al.* (2010). We suggest several biotic and abiotic factors that may nullify under-vine cover crop influences on inoculum, with the most likely being the presence of mid-row vegetation and the grapevines themselves – both of which may provide their own source of AM inoculum, buffering against treatments where plants were absent (HC and TM).

5.4.4 *Mid-row and grapevine inoculum, no-till practice and future studies*

Although the experimental treatments considered here did not result in impacts on colonisation, there are other vineyard practices that may have influenced results. Within our trial it is likely that additional sources of AMF inoculum were present – buffered by vegetation in the mid-row and indeed the grapevines themselves. The relatively high AMF inoculum potential in soil denuded of under-vine vegetation could also be explained by the presence of grapevine roots (Holland *et al.* 2014; Radić *et al.* 2012; Schreiner & Mihara 2009; Valentine *et al.* 2006). The constant presence of a mycorrhizal-dependent species is a significant contributing factor to the replenishment of propagules and soil inoculum buffering capacity. Moreover, the mid-row may offer a source of AM inoculum, with a dense sward cover increasing AMF propagules from mid-row to under-vine. One study found hyphae – and spore propagules – extending some 30 cm (in the extreme) beyond the host root system (Harinikumar & Bagyaraj 1995). Given the width of the under-vine area is approximately 60 cm and is bordered either side by mid-row sward, it is reasonable to suggest mid-row impact on under-vine inoculum potential is not insubstantial. Further studies reached a similar conclusion, suggestive that cover crops (species-specific) increase fungal diversity and play pivotal roles in certain soil processes (Trouvelot *et al.* 2015; Vukicevich *et al.* 2019a). Other authors suggest that while cover crops may increase fungal diversity, they appear to be a less dominant factor than the mere presence of perennial grapevines in the enhancement of mid-row inoculum potential (Baumgartner *et al.* 2010; Trouvelot *et al.* 2015).

One particularly pertinent variable that unites all treatments is the use of a *no-till* management practice. Evidence for tillage impacting negatively on AMF diversity and function is relatively consistent in literature, especially studies involving long-term field trials (Brito *et al.* 2012). A comparative analysis of tillage vs no tillage on a southwest German vineyard system found a marked decline in AMF spore density and fungal diversity in tillage treatments (Fritz & Bruno 2018). Where the literature appears inconsistent is in discussions surrounding specific propagule damage – spore vs hyphal fragments. Kabir (2005) highlights several key sources pertaining to AMF decline through tillage, however, through their own study, suggested that while AMF spores may withstand tillage, hyphae fare poorly. Soils from our trial site saw a no-till practice (after treatments were established), leaving both hyphae and spores undisturbed. Sieving of soils prior to glasshouse experimentation may have impacted poorly on hyphal fragments, however, it is likely that fungal spores remained dormant (Smith & Read 2008). Incubation of soils prior to each planting was likely to activate AMF spores, enabling significant root colonisation throughout all treatments (Schimel *et al.* 2007). We did not directly test the effects of tillage vs no tillage on our treatments, and although vineyards remain relatively undisturbed during their working lives, changes in management practice and grapevine rootstock are important instances of potential disturbance. To further test the hypothesis that soil disturbance (or a lack thereof) is a dominant factor in AMF inoculum potential, we would propose a future, multifactorial trial that compared under-vine treatments in a till vs no-till study (i.e. herbicide till vs herbicide no till vs cover crop till vs cover crop no till).

To more accurately determine which vegetative feature is the dominant source of inoculum (if any), one could test the influence of mid-row vegetation on under-vine inoculum potential either by: 1) denuding the mid-row or, 2) inserting artificial barriers between the mid-row and under-vine; in each case eliminating mid-row influence such that only grapevines and under-vine cover crops are potential sources of inoculum. The direct effect of host presence appears pivotal to AM persistence via inoculation, however, it is also worth noting the indirect effects of disturbance, or in this case, a lack thereof.

5.4.5 *Implications for under-vine management*

The implications of these results in terms of future under-vine management practices are numerous, owing to the multifarious influences of under-vine cover-cropping. Pertaining directly to AMF inoculum potential, the results appear uncertain as to the direct influence of different management practices. Sources of inoculum in the vineyard can be numerous, with mid-row and grapevine roots offering potential buffering capacity – aiding in the replenishment of AM spores. All of this aside, however, it should be noted that we are discussing inoculum *potential* – that is to say, the *potential* for plant-AM colonisation, which is high between all treatments, however, this is only possible in the presence of active plant roots. We have noted previously that increased AM hyphal networks are beneficial to soil fertility in numerous ways – more than simply nutrient acquisition for the focal crop (grapevines). Therefore, although AM inoculum (notably spores) may withstand herbicide application, they still require plant root systems in order to establish hyphal networks. Given this, we may suggest that establishing under-vine cover is important to allowing these networks to form. Results from this study showed conclusively that not all plant roots are colonised to the same extent (e.g. medic vs ryegrass) and therefore, we may further suggest that planting a mycotrophic crop such as medic (*M. truncatula*) could be more beneficial to establishing hyphal networks than a less mycotrophic species, or for that matter, nothing at all.

Acknowledgements

It is important to acknowledge that we conduct ecological field trials and indeed our entire investigative process on traditional land. Therefore, we acknowledge and pay respect to the traditional owners of Langhorne Creek: the Ngarrindjeri people and the land upon which the Waite Institute is situated: the Kaurna people. Furthermore, as representatives of The University of Adelaide, we acknowledge and thank Wine Australia for their ongoing support and funding. Joseph Marks is also the recipient of the *Dr Tony Jordan OAM Award* (scholarship), funded by Wine Australia (project code: UA1706).

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Chapter Six: Home or away?

6 Home or away: decomposition of under-vine cover crops is unaffected by *home-field advantage*

This was the first hypothesis and experimental design that I conceived entirely on my own.

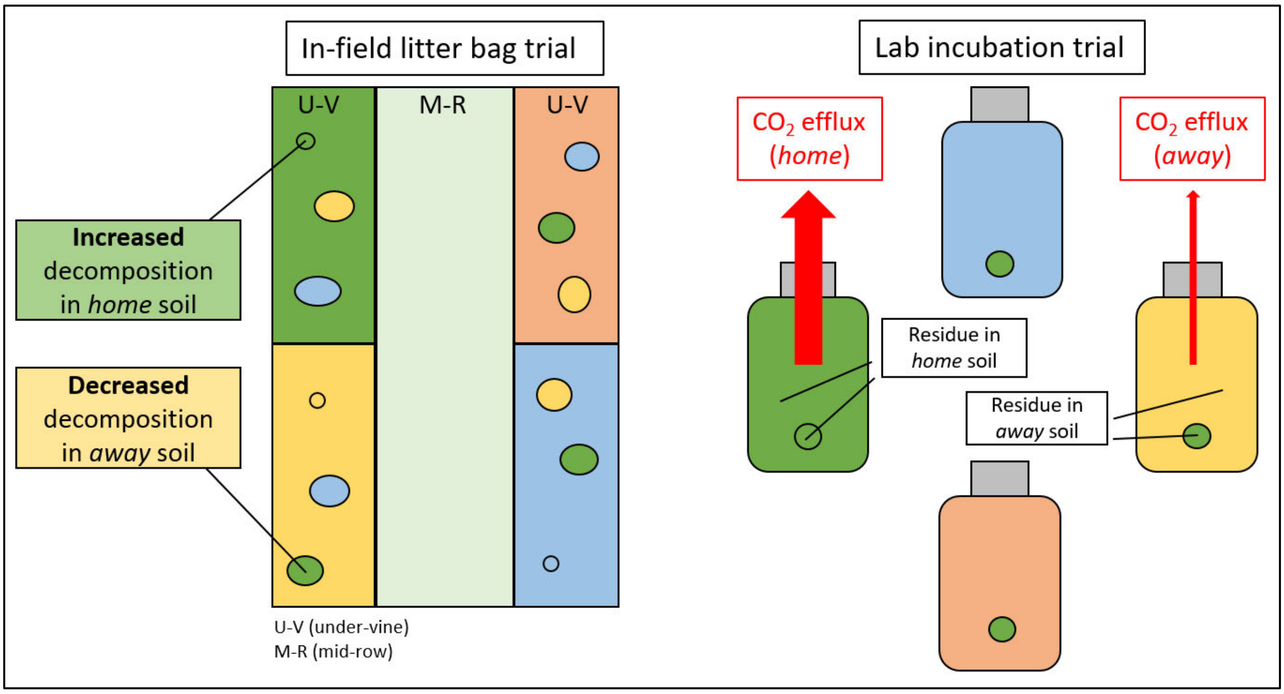
The idea came from attempting to mitigate potential home-field advantage in Exp. 1 (AMF).

I thought "why not make home-field advantage in vineyards a hypothesis in itself".

I think decomposition is a beautiful process – both the end and the beginning of life.

Why not see how it operates underneath grapevines?

The diagram below is a conceptual model of home-field advantage affecting decomposition.



Statement of Authorship

Title of Paper	Home or away: decomposition of under-vine cover crops is unaffected by *home-field advantage.*
Publication Status	☐ Published ✓ Submitted for Publication ☐ Accepted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	This manuscript has been submitted to the journal Plant and Soil.

Principal Author

Name of Principal Author (Candidate)	Joseph Marks		
Contribution to the Paper	Conceptualisation, data retrieval, data analysis, knowledge, drafting & editing.		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	20/04/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr Thomas Lines		
Contribution to the Paper	Conceptualisation, data retrieval, data analysis, knowledge, drafting & editing.		
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Abstract

The use of cover crops in vineyards is becoming an increasingly-adopted strategy for mid-row and under-vine management. Understanding how different species and combinations of cover crop litter decompose can have far-reaching consequences for carbon turnover and nutrient cycling in both vineyards and other agroecosystems. Plant litter decomposition may be affected by several ecological mechanisms, including *home-field advantage* and *substrate matrix interactions*. The former postulates that plant litter will decompose faster and more completely in the same soil from which it originates, while the latter suggests that decomposition is driven less by species-specificity and more by physicochemical characteristics. To test which interaction may be primarily responsible for decomposition, we undertook two experiments, one in in-field and one in a controlled laboratory incubation. Litter bags containing cover crop residues were carefully weighed and buried under both their own litter type and every other litter type, before being exhumed and re-weighed to test for biomass loss. The same experimental design was applied to the laboratory incubation, with total accumulated CO_2 measured as an indicator of microbial activity to determine whether *home-field* microbes favoured their own litter versus another. In both experiments we were unable to determine any significant ($p > 0.05$) differences in either biomass loss or total accumulated CO_2 between treatments. Owing to these results, we must conclude that the *home-field advantage* hypothesis may not be influence decomposition in vineyard systems to the same extent that it might in more disturbed systems.

6.1 Introduction

Understanding decomposition is of primary importance when quantifying both the rate and totality to which various plant residues cycle nutrients and carbon back into soil systems. Decomposition is dependent on multiple biotic and abiotic variables, not least; C:N ratio, lignin content soil moisture and organic matter processing (Bachega *et al.* 2016; Chassain *et al.* 2021; Ng *et al.* 2015). The dynamics of decomposition are complicated and can vary dramatically between ecosystems. One aspect of ecological theory posits that plant residues falling or becoming buried beneath their own living biomass decompose more rapidly and completely than under a foreign biomass – the so-called home-field advantage hypothesis (or HFA) (Austin *et al.* 2014; Perez *et al.* 2013). Studies on the effects of HFA suggest that certain decomposers (bacteria, fungi and macro-fauna) are adapted to specific residue types, through specialised enzyme production and niche recourse-affinity (Austin *et al.* 2014). A simpler theory – though of a similar nature – is the substrate matrix interaction hypothesis (SMI). Under this revised theory, residue decomposition (*home* vs *away*) operates on a spectrum of similarity, with residues of similar physicochemical nature able to decompose more rapidly and completely within their reciprocal systems (Freschet *et al.* 2012). Literature suggests that HFA is more pronounced in more frequently disturbed ecosystems. (Gießelmann *et al.* 2011). Understanding, therefore, if HFA influences decomposition in heterogeneous or cultivated systems, such as vineyards is less well understood.

In recent years there has been a paradigm shift regarding vineyard floor management, with the adoption of less invasive practices (Ruiz-Colmenero *et al.* 2013). The use of mid-row cover crops has long been practiced in viticulture; however, less research has been conducted on the effects of under-vine management (Ball *et al.* 2020; Karl *et al.* 2016). Sowing cover crops under-vine (and mid-row) reduces the reliance on herbicides and offers numerous potential benefits to both the focal crop (grapevines) and the vineyard ecosystem in general (Peregrina *et al.* 2012; Steenwerth & Belina 2008). Applying ecological theory to vineyard floor management can provide valuable insight into how various cover crop and mulch residues decompose and cycle nutrients. Furthermore, applying theories of HFA and SMI to under-vine decomposition may help inform on vineyard floor management practice in relation to successional planting of cover crops (Brunetto *et al.* 2011; Ferreira *et al.* 2014).

Within a vineyard trial site in the Langhorne Creek region of South Australia, four under-vine treatments were selected to test whether HFA and/or SMI influence the decomposition of three under-vine residues. To test whether HFA or SMI influence decomposition under-vine, we propose to take samples from three under-vine residues – straw mulch, wallaby grass and a medic/ryegrass combination – and transplant them into soil under *both* their own derivative biomass *and* every other, including a herbicide-managed control. The influence of HFA appears to be more pronounced in recalcitrant residues as they require more specialised enzymes to degrade (Fanin *et al.* 2021). Thus, we hypothesise that residues of straw mulch and wallaby grass will be more prone to HFA effects, owing to their lower C:N ratio and higher lignin content. To adequately test this hypothesis, we aim to engage both an in-field litter bag trial and a laboratory-controlled incubation trial. In this, we aim to cross-validate our hypothesis by testing decomposition as a function of biomass loss (in-field litter bag) and as a function of microbial respiration (laboratory incubation).

6.2 Materials and Methods

The long-term influences of home-field advantage (HFA) in under-vine residue decomposition were assessed over two phases of experimentation: the first was conducted in the field, while the second was conducted in the laboratory. Experimentation used residues and soils from a long-term vineyard trial site at Langhorne Creek (LC, hereafter) in South Australia (35.302”S, 138.952”E). The trial itself was established in 2014, with seven under-vine treatments including five cover crop combinations, a straw mulch and herbicide-managed control (Penfold *et al.* 2015). The present study focused on four of the seven under-vine treatments, namely: *Lolium* and *Medicago* grown under-vine (medic and rye grass or M+R, hereafter); *Rytidosperma caespitosa* grown under-vine (wallaby grass or WG, hereafter); Triticale mulch applied under-vine (TM, hereafter) and; a plant-free herbicide-managed, under-vine control (herbicide control or HC, hereafter). See Table 1 for a detailed summary of under-vine treatments. Within the trial site, under-vine treatments were randomly assigned to four blocks in a randomised complete block design (RCDB), with each treatment present with each block to account for underlying spatial heterogeneity at the site level. Each treatment plot comprised nine grapevines (or three panels), with a small buffer zone – consisting of one panel – surrounding each treatment and the trial zone perimeter to account for potential spatial edge effects. The mid-row was maintained by the site manager, with regular mowing throughout the year and planted with non-grass or leguminous species.

Table 1. List of four experimental treatments. Each treatment represents one under-vine management practice implemented at a trial site in Langhorne Creek, South Australia. Listed is the treatment code (abbreviated name), common name, species/cultivar name and specific properties (as utilised in vineyard management). Henceforth, treatments codes will be used, i.e. HC = Herbicide control; TM = Triticale mulch; M+R = Medic + rye grass and; WG = Wallaby Grass.

Treatment code	Common name	Species/cultivar name	Properties
HC	Herbicide control		Traditional management practice, vegetation cover suppressed. Herbicide used was glyphosate (during vine dormancy) and ammonium glufosinate (when vines were active).
TM	Triticale mulch		Soil stabilisation; temperature regulation and; soil moisture enhancement. Hand-laid (50 t ha^{-1}).
M+R	Safeguard ryegrass / Scimitar	*Lolium rigidum / Medicago polymorpha*	Annual grasses; fibrous roots for soil stabilisation; water infiltration potential; high N-uptake therefore grown in combination with medic (N-fixer).
WG	Wallaby grass	*Rytidosperma caespitosa*	Native grass with adaptive traits to water-reduction; weed-suppression and; increased native microbial biodiversity (Mitchell 2003)

6.2.1 *Litter bag trial and experimental design*

The litter bag trial was conducted during September and October of 2020 (Austral spring) to coincide with bud burst, and to allow ample soil moisture to promote decomposition of plant residues. The experimental design of the trial was conceived to allow the burial of three under-vine residues (M+R, WG and TM) in soil beneath four under-vine treatments (HC, M+R, WG and TM), i.e. buried under their own treatment plot (home-field) and under other treatment plots (*away*). Burial of litter bags adhered to the RCB design of the vineyard trial and thus residue (litter bags) from block one were only buried beneath under-vine treatments within block one and the same for blocks two, three and four. E.g. WG from block was sampled and divided into four litter bags and buried under WG, M+R, TM and HC from block one, etc. (see Fig. 1 for detailed schematic).

Table 2. List of vineyard (floor and grapevine) properties and management practices used at the trial site in Langhorne Creek, South Australia.

Vineyard properties	**Langhorne Creek vineyard**
Grape varietal	Cabernet Sauvignon
Vine age	21 years
Rootstock type	Own (Cab LC-10)
Rooting depth	~300-400 cm
Training method	Single cordon trellis
Pruning method	Saw box pruned
	(15-18 t ha^{-1})
Pest/disease management	Occasional foliar fungicide
Mid-row management	Slashing and heavy rolling
Soil type	Brown Sodosol
Irrigation regime	3.5 ML ha^{-1}
Dripper spacing	2.5 m row^{-1}

Residues were sampled from the vineyard trial site using a quadrat placed randomly within each panel (three panels per treatment plot), with residues bagged and kept separately. Thus, there were four bags of fresh residue per treatment plot, each representing one of four blocks (n=12 total). Prior to experimentation, residues were oven dried at 40°C, with subsamples ground to a fine powder using a ball-bearing mill (MM400, RETSCH, Haan, Germany). Composite samples of each residue were sent to the Australian Precision Agricultural Laboratory (APAL) in South Australia for analysis of carbon (total and organic) and total nitrogen. Carbon and nutrient analyses would serve for use during both phases of experimentation. The remaining residues were hand-cut using scissors to 1-2 mm segments and each residue was allocated to a new "clean" bag prior to litter bag construction. Litter bags were constructed using 25 µm mesh, with each bag cut to the same dimension (50 x 50 mm). Residues were placed in their respective litter bags, along with a unique identifier and sealed using a laminate heat sealer. All air-dried litter weights were recorded, along with bag weights and other apparel. At the culmination of bagging the residues there were 48 litter bags. Burying litter bags involved hammering a core (100 mm × 100 mm) into soil under-vine, removing the soil core and placing the litter bag at the bottom, before replacing the soil core. To identify the litter bags upon completion of the trial, coloured string was attached and anchored to a peg aboveground. Retrieval of the litter bags occurred eight weeks post-burial. In the laboratory, litter bags were oven-dried at 40ºC (to account for residue moisture mass) and carefully opened. The mass of each residue was recorded and deducted from the initial, pre-burial mass.

Table 3. List of physicochemical properties for under-vine treatments (soil and cover crop/straw mulch). Samples were composites formed from within each treatment block and averaged across four blocks (replicates). Soil samples were taken from 0-10 cm depth. Values are mean ± SE.

	Soil treatments (Langhorne Creek)							
Soil properties	HC		TM		M+R		WG	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Nitrate (mg kg^{-1})	6.58	1.09	10.03	0.37	12.28	1.53	13.4	2.12
Ammonium (mg kg^{-1})	NA	NA	0.25	0.25	1.70	0.63	0.90	0.53
Total nitrogen (%)	0.19	0.01	0.20	0.01	0.22	0.02	0.20	0.01
Organic carbon (%)	0.88	0.06	1.21	0.13	1.35	0.19	1.22	0.09
CC residue properties			TM		M+R		WG	
			Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Total nitrogen (%)			1	0.0	3.75	0.25	1	0.0
Organic carbon (%)			42	1.0	42	0.75	42.5	0.29
C:N ratio			42:1	1.0	12:1	0.75	43:1	0.29

6.2.2 *Residue incubation trial and experimental design*

The residue incubation trial mirrored the under-vine litter bag trial and served to reduce in-field variation by imposing laboratory condition and reduce inference by abiotic (vineyard) conditions (see Fig. 1 for detailed schematic). Soils were sampled under-vine from the LC vineyard using a core (100 mm × 100 mm). Soil samples were taken from treatments of HC, M+R, WG and TM across four blocks and kept separately (n=16 samples). Soils were air-dried at 40ºC and sieved to remove >2mm large organic matter and gravel content. Prior to incubation, samples were sent to the Australian Precision Agricultural Laboratory (APAL) for basic nutrient analyses and total organic carbon content. In addition to this, soils were also tested for gravimetric water content (GWC) and water holding capacity (WHC) using the hanging water column method as described by Cavagnaro (2016). There were no significant differences in WHC between soil treatments (p >0.05), therefore WHC (g soil / g water) was averaged between treatments. Soil samples of 50 g (adjusted for GWC) were weighed into 250 mL incubation jars and brought up to 50% of WHC, before being incubated for a period of seven days to promote microbial activity (Liu *et al.* 2019; Penfold *et al.* 2015; Stirling *et al.* 2019). There were no significant differences in organic C content between under-vine residues, therefore each incubation chamber was amended with 10 g kg^{-1} (or 1%) of residue dry mass and mixed thoroughly. Mirroring in-field experimentation, soils were partitioned by block and each treatment was divided four times and amended with residue (*home* and *away*). Soil respiration was analysed using an LI-820 CO_2 gas analyser (LiCor, NC) at days 1, 2, 3, 5, 7, 9, 11, 14, 17, 21, 25, 30, 37, 45, 56 and 78. Prior to CO_2 analysis, the headspace was flushed with air (of a known CO_2 concentration) to remove residual CO_2 from the headspace, before being sealed and incubated at room temperature for 30 minutes.

6.2.3 *Statistical analyses*

Prior to significance testing all treatment variables were tested for normality and homoscedasticity using the Wilkes-Shapiro test (R Studio version 3.6.3). Those data that were found to be non-normal in distribution were transformed. Litter bag data were analysed based on biomass loss (g) as function of decomposition. Analysis of biomass loss was conducted between residue treatments, that is, M+R residue decomposition was only compared to other M+R residue decomposition within different under-vine soils, e.g. M+R residue in M+R soil *vs* M+R residue in WG soil *vs* M+R residue in HC soil etc. Two-way ANOVA was used to test for significant differences in biomass loss, with both *treatment* and *block* as factors. Boxplots were constructed to visualise data from each soil treatment and were facet wrapped using ggplot2 in R Studio to visually compare biomass loss between treatment groups (Wickham 2009).

Respiration data were processed and converted to account for mass of soil ($C\text{-}CO_2$. kg soil^{-1}. Hr^{-1}), with each treatment/replicate peak summed to achieve a cumulative respiration at time 78 days. Significance testing was undertaken using two-way ANOVA and compared between treatments of like residue, as described previously. Total cumulative respiration (as a function of decomposition) was compared between treatments to determine if there was a significant difference in total respiration between residues within different soils. Boxplots were constructed to visualise data from each soil treatment and were facet wrapped using ggplot2 in R Studio to visually compare total accumulated respiration between treatment groups. Cumulative growth curves were constructed using ggplot2 in R Studio, with the geom_line function highlighting 95% confidence ellipses. All data were analysed and visualised using R open source statistical software (version 1.2.5033), with additional software packages “agricolae”, “ggplot2”, “ggfortify” and “tidyverse”. Alpha was set at 0.05 (De Mendiburu 2014; Horikoshi & Tang 2018; Wickham *et al.* 2019).

6.3 Results

6.3.1 *Litter bag decomposition unchanged in home vs away burial sites*

Cover crop and straw mulch residue decomposition were measured as a function of biomass loss from 25 µm mesh litter bags (Fig. 2). Biomass loss was compared between treatments in a multifactorial analysis, where each residue was buried in soil beneath each under-vine treatment (including its own) to test whether HFA exists between under-vine residues and their soil microbial assemblage. Two-way ANOVAs were conducted to test for significance in biomass loss between like-residues buried beneath different under-vine treatments (seven under-vine treatments per block). Statistical testing revealed no significant difference in mean biomass loss between treatments ($p < 0.05$). This suggests that, over a given period of time, under-vine residue decomposition appears unaffected by burial location. It is therefore suggested that HFA did not exist between under-vine soil microbial assemblages and their host residue (i.e. M+R residue decomposes to the same extent when buried beneath *any* under-vine treatment, including its own living biomass). Although we did not find sufficient evidence for a home-field advantage, we did observe patterns of difference in decomposition between residue types, as would be expected, owing to differences in C:N ratios. In this, we found that medic + ryegrass residue decomposed to a far greater extent than either wallaby grass or straw mulch.

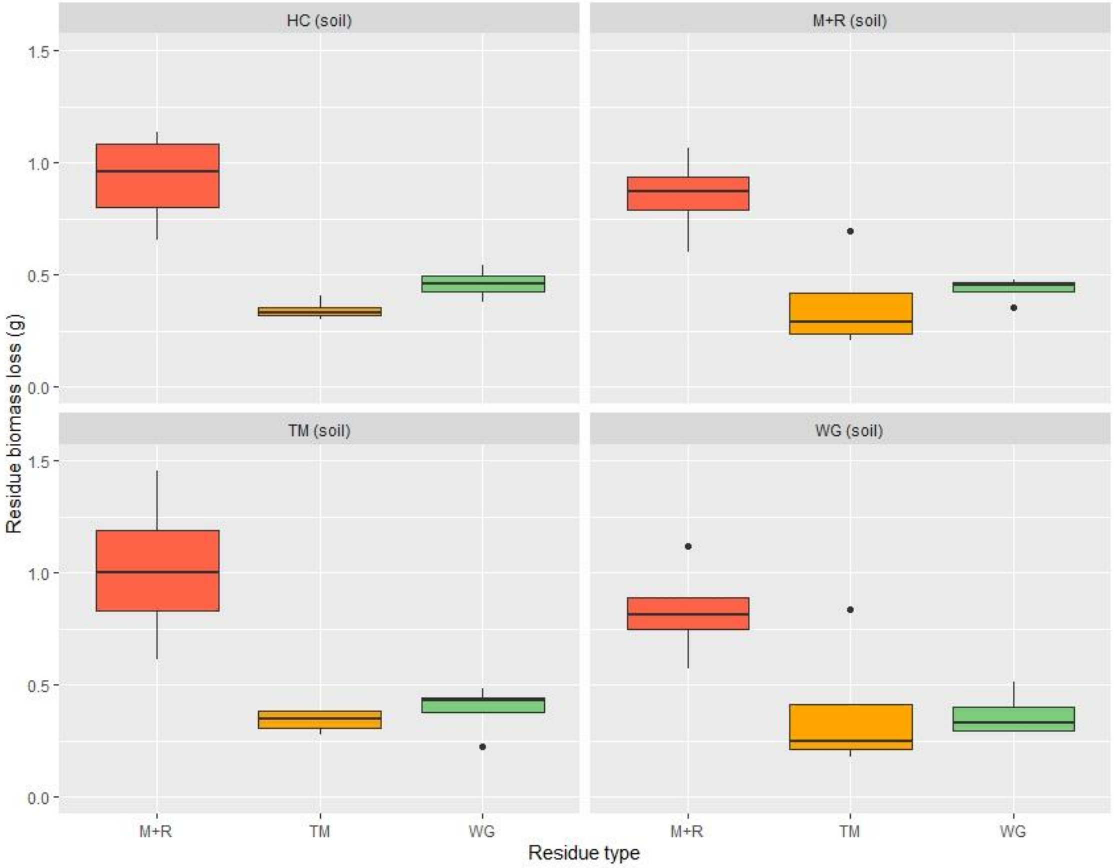

Figure 2. Boxplots showing median values for total biomass loss (g) for under-vine residues: medic + rye (M+R); mulch (TM) and; wallaby grass (WG) when buried in litter bags under treatments of herbicide (HC); M+R; TM and; WG. Litter bags are buried under home-field treatments, as well as other treatments to understand if decomposition rates are greater in home-field soil than *away* soil. There were no significant differences in home vs *away* soil for any of the under-vine residues (p<0.05). Boxplots are facet wrapped by burial site, with decomposing residues shown in contrasting colours (ggplot2 in R Studio).

Table 4. Mean biomass loss from 25µm litter bags filled with residues from treatments of TM, M+R and WG and buried underneath under-vine treatments of HC, TM, M+R and WG. Values are mean ± SE.

Soil (treatment burial site)

CC residue biomass loss (g)	HC		TM		M+R		WG	
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
TM	0.34	0.02	0.34	0.03	0.37	0.11	0.38	0.15
M+R	0.93	0.11	1.02	0.18	0.85	0.10	0.8	0.11
WG	0.46	0.04	0.39	0.06	0.44	0.03	0.37	0.05

6.3.2 *Microbial preference appears non-specific for home vs away residues*

After 72 days of incubation, total accumulated CO_2 compared between treatments as a proxy for microbial activity and residue decomposition. There was no significant difference in the total amount of CO_2 respired between treatments ($p < 0.05$). That is to say, microbial preference appeared non-specific to home-field residues.

Table 5. Total accumulated CO_2 efflux from treatments of under-vine soil (HC, TM, M+R and WG) when amended with fine-chopped residues of TM, M+R, WG and B (blank control). Values are mean ± SE.

Soil (treatment burial site)

CC residue efflux	HC		TM		M+R		WG	
(mg C-CO_2 kg soil^{-1})	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
TM	18026.9	1033.8	14069.1	1576.2	16932.3	2673.4	16815.4	1686.6
M+R	12225.4	477.9	11062.3	830.9	12956.6	152.3	11300.1	319.3
WG	11085.1	1700.2	11806.7	747.6	14597.1	1304.0	12180.7	1522.9
B	3431.3	297.2	3494.9	367.1	5074.8	928.2	4122.2	201.2

Rather, decomposition and microbial activity appeared driven by the inherent physicochemical characteristics of the various under-vine residues. This is particularly evident in Fig. 3, where accumulation of CO_2 evolution adhere firmly to residue type, rather than the soil in which they were incubated. Patterns of CO_2 evolution (Fig. 4) are also closely aligned by residue type, rather than home-field soil. Non-linear modelling of CO_2 evolution (Fig. 4) shows distinct patterns of residue decomposition, however, these patterns appear to be a function of plant residues and not a treatment-based result. These data validate those results shown in the previous litter bag experiment, with results showing that HFA may not be the prevailing factor in under-vine decomposition and nutrient cycling.

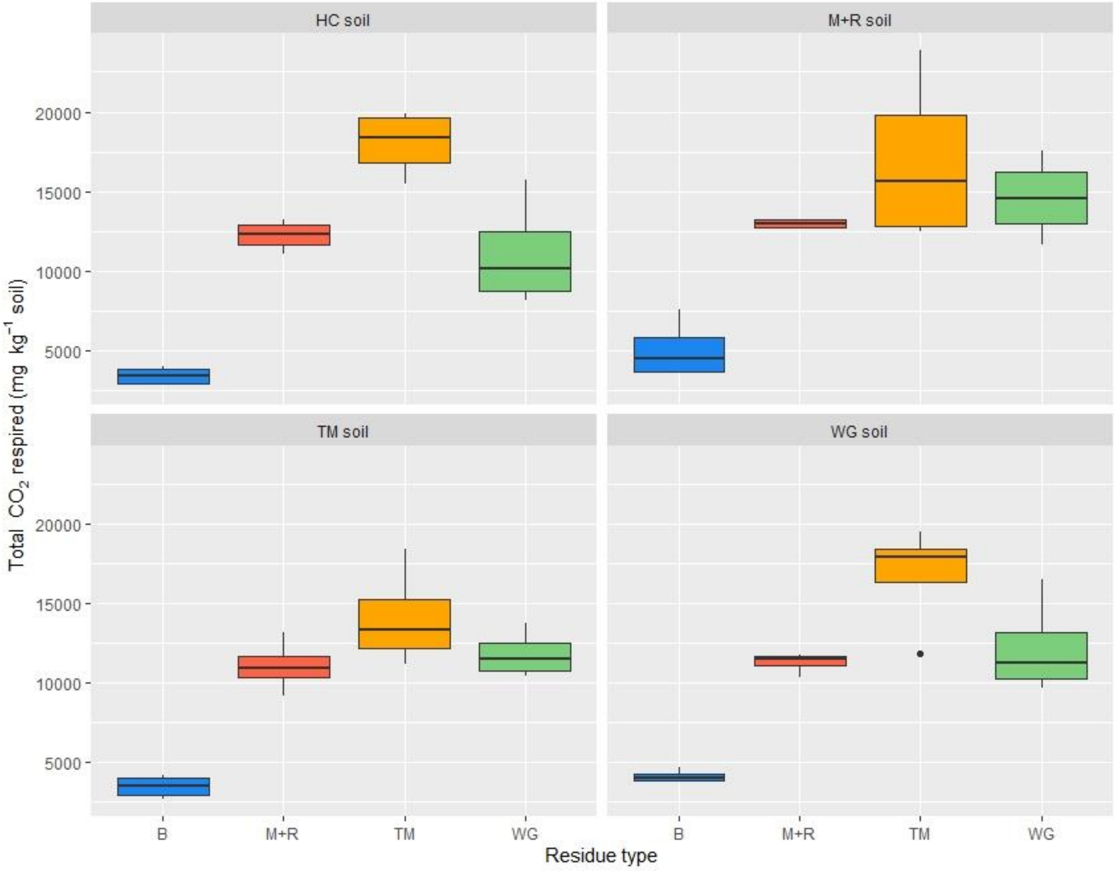

Figure 3. Boxplots showing median values for total accumulated CO_2 (mg C kg^{-1} soil) for under-vine residues (including a blank control). Residues were mixed into samples of under-vine soil sampled from beneath their own residue (home-field) and other under-vine treatments (*away* soil) to understand if decomposition was greater in home-field soil than *away* soil. There were no significant differences in home vs *away* soil for any of the under-vine residues ($p<0.05$). Boxplots are facet wrapped by burial site, with decomposing residues shown in contrasting colours (ggplot2 in R Studio).

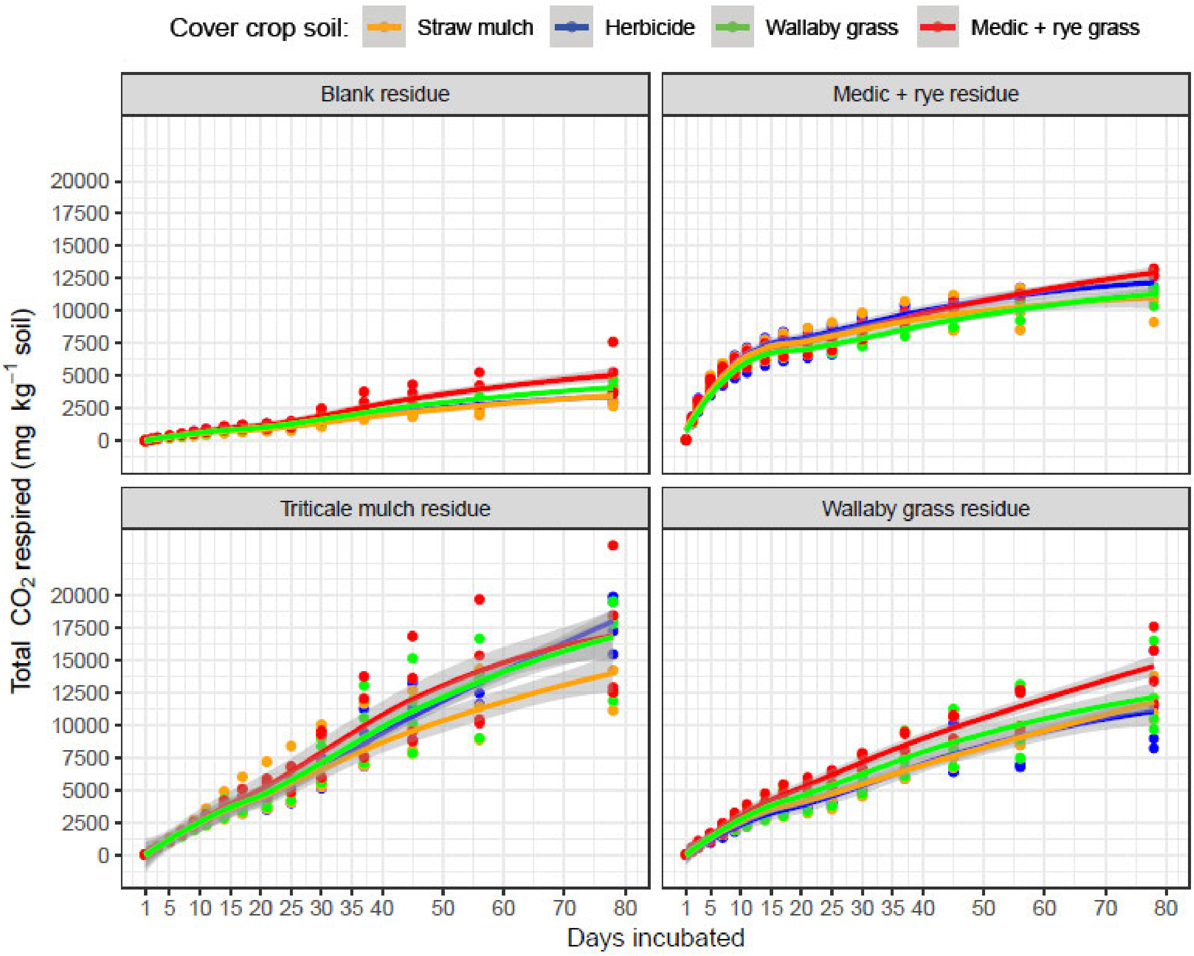

Figure 4. Scatterplots of modelled CO$_2$ respiration data sampled at several time points (max = 78 days). Soils were sampled from beneath under-vine treatments and amended with residues (home-field and away). Scatterplots are facet wrapped by residue type, demonstrating observable differences in residue decomposition when incubated in home-field soils and away soils. In contrast to Figs. 2 and 3, Fig. 4 is facet wrapped (or grouped) by residue type, rather than soil type as this more accurately depicts decomposition patterns in under-vine soils. There were no significant differences in total accumulated CO$_2$ efflux from soils amended with home-field residues vs foreign residues ($p<0.05$). Scatterplots were constructed using ggplot2 and ggfortify in R Studio.

6.4 Discussion

The purpose of this study was to test the initial hypothesis that under-vine cover crop residues decompose more rapidly and completely in the soil under which their derivative plant was grown (*home*) versus a different soil (*away*). Decomposition was quantified as a function of residue biomass loss (field) and total microbial respiration (laboratory). In both cases, we were unable to distinguish any significant difference in either the rate of decomposition or totality of decomposition and are thus forced to surmise that home-field advantage (HFA) is not the dominant factor in under-vine decomposition in vineyard systems.

Although the above results stand against our initial hypothesis, literature pertaining to previous studies is varied on the effects of HFA, especially in cultivated or disturbed systems, such as vineyards (Austin *et al.* 2014; Gießelmann *et al.* 2011). Moreover, Gießelmann *et al.* (2011) suggest that HFA effects may be less observable in areas of higher plant diversity and turnover and thus where decomposer specificity at the microsite level is less clearly defined. Indeed, much of the current literature suggests that HFA is most pronounced in perennial systems with either a single dominant species or in those comprised of residues of distinct physicochemical nature (Austin *et al.* 2014; Ayres *et al.* 2009). Such understandings of the effects of HFA on decomposition align well with our own findings. The vineyard trial site is sporadically disturbed with mid-row machinery and human traffic. Moreover, it is a system that is heterogeneous in residue type, with mid-row grass cover and perennial grapevines (including annual vegetative biomass) present within (and between) all under-vine treatments. These factors may dilute HFA effects at the microsite level, either through microbial dispersal by humans and machines or – perhaps more likely – through the proliferation of generalist decomposer communities, owing to similar residue signatures entering the system via multiple sources (vine leaves, canes, etc.).

The effects of HFA are thought to be more pronounced in the decomposition of recalcitrant residues, owing to the requirement of specialist decomposers and specific enzymes to degrade low quality or more complex compounds (Milcu & Manning 2011; Wallenstein *et al.* 2013). In this, we may hypothesise that residues of TM and WG would show more pronounced effects of

HFA than M+R residue, given their higher C:N ratios and greater concentration of lignified compounds (Table. 3). This, however, was not observed either in biomass loss (Fig. 2), total accumulated CO_2 (Fig. 3), nor in patterns of decomposition over time (Fig. 4). The intrinsic nature of each residue’s decomposition was observed in Fig. 4, with M+R residues showing a sharp initial rise in decomposition, owing to its low C:N ratio and increased supply of nitrate for microbial activity (Table. 3). Both TM and WG residues maintained a similar pattern of decomposition, with a slow initial spike, followed by a steady-state pattern as decomposition curves reached an asymptote and maintained a steady rate of decomposition thereafter – patterns likely indicative of more recalcitrant materials. These patterns, although unique to each material (intrinsic plant phenology), did not show significant differences within the specific soil they were incubated (i.e. no HFA effect).

The HFA hypothesis provides a useful explanation for decomposition patterns within certain ecosystems, however, it may be expounded upon to better understand patterns within more complex or heterogeneous systems (Freschet *et al.* 2012; Veen *et al.* 2015). The substrate matrix interaction (SMI) hypothesis suggests that, rather than decomposers developing species-specific affinities for particular residues, they become adapted to general residue types based on broader physicochemical signatures (Veen *et al.* 2015). Under this hypothesis, residues should decompose at higher rates and more completely when transplanted into ecosystems where the derivative biomass is of a similar type (Austin *et al.* 2014; Freschet *et al.* 2012).

Contrasting these two hypotheses, we suggest that SMI may be more applicable in heterogeneous systems such as vineyards, where multiple litter types merge in the under-vine and mid-row. If we apply this concept to our study, we might expect that recalcitrant residues (TM and WG) would decompose more completely in each other’s derivative soil, owing to their similar C:N ratio. Following this line of inquiry, we might also expect to see both recalcitrant residues decompose to a lesser extent in M+R soil – the suggestion being that decomposers under M+R residues are less adapted to lower levels of N and thus cannot decompose low quality residues as adeptly (Gartner & Cardon 2004; Perez *et al.* 2013). If the SMI hypothesis were more applicable to our results we would expect to see significant differences in biomass loss and CO_2 evolution (Figs. 2 and 3) in residues of TM and WG in soils from under TM and WG versus soils of HC and M+R. We would also expect to see differences in patterns of decomposition (Fig. 4), with the decomposition of more recalcitrant compounds presenting a steeper steady-state past the point of asymptote. In both cases, however, we did not observe any significant differences, nor any adversely contrasting patterns of decomposition between any residues in their *home* soil versus *away* soil. From this, it is reasonable to suggest that neither HFA, nor SMI appear to influence residue decomposition under-vine.

6.5 Conclusion

We did not find sufficient evidence to suggest that either home-field advantage or substrate matrix interactions significantly influence under-vine residue decomposition in vineyard ecosystems. Evidence from previous studies bolster this result by suggesting that HFA is most applicable to homogenous, undisturbed systems where the decomposer community has become strongly adapted to a niche resource base. Although no treatment effect was found, we did find that residue type affects decomposition, with residues of medic + ryegrass decomposing at a higher rate than those of straw mulch or wallaby grass, owing to differences in C:N ratio. That there was no significant treatment effect suggests that vineyard decomposers are less selective in their ability to degrade different residue types. This may be due to several factors, including heterogeneity of residue type and frequency of disturbance by human and machine traffic, as well as the presence of a diverse mid-row between all treatments. These results suggest that vineyard floor systems are heterogeneous in both their decomposer assemblage and plant residues. This functional redundancy among decomposers is beneficial to vineyard floor management as it demonstrates that nutrient cycling through residue turnover is likely unaffected by successional under-vine practices.

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Chapter Seven: A General Discussion

7 A General Discussion

I have a tendency to think in medical terminology when stitching this thesis together. This being a thesis-by-publication (or a hybrid thereof), it is a compendium of hypothesis-testing and subsequent manuscripts. The thesis whole, therefore, should aim to link these vital organs – coalescing them into a functioning body. I like to think of the linkages as fascia or some kind of interstitial fluid – the unsung structures holding the vital organs in place. I hope, by the conclusion of this thesis, I have succeeded in weaving together a distinct narrative – a body. So, to begin the end, let us examine and tie these seemingly disparate organs together, recapping and synthesising each, both separately and together, before finally ending with more questions than answers; because after all, this is science…

7.1 The whole body

The central premise of this project has been to investigate how alternative under-vine management practices (namely, cover crops) influence the ecological intensification of vineyard floors. Where other projects may have focused more directly on the grapevines themselves, this investigation has taken a different approach. My academic background lies in soil ecology, rather than viticulture and to this end I have sought to apply ecological thinking to this investigation. In this, I have viewed the vineyard as an ecosystem in and of itself, relegating the grapevine to a simple biotic constant (present within each treatment). *Ecological intensification*, in this sense, posits that by transitioning vineyards into natural systems they are able to equilibrate and more sustainably 'self-manage'. As evidence suggests, the implementation of under-vine (and whole-floor) cover crops can enhance vineyard ecosystem services, thus decreasing the need for interventional management, such as fertilizer and herbicide inputs. As we have observed, *specificity* is essential and, through the exploration of seven treatment effects (five cover crop combinations, a surface mulch and herbicide control), we have demonstrated that several key biotic and abiotic factors drive the selection process. This project has therefore sought to observe and quantify some of the multifaceted ecosystem services of under-vine cover crops:

1. Symbiotic partnerships with mutualistic arbuscular mycorrhizal fungi – a crucial ecosystem service for many terrestrial plants and one that enhances nutrient (P and Zn) cycling dynamics. In other studies, AMF have been shown to play a role in increasing soil-plant pathogen resistance and enhancing SOC dynamics through decomposition and organic biomass accumulation (Keller 2015; Smith & Read 2008).
2. Soil organic carbon (accumulation, retention and turnover) – another crucial ecosystem service provider, both directly and indirectly. Soil organic carbon provides numerous physicochemical benefits to vineyard soil, key among them enhancing soil aggregate stability and soil water dynamics (Ruiz-Colmenero *et al.* 2013). Like AMF, SOC can provide a marker for ecosystem health and soil fertility.
3. Decomposition dynamics are closely related to both AMF function and SOC turnover – this is the third ecosystem service explored within this body of work. Cover crop decomposition, C turnover and nutrient cycling are processes pivotal to the establishment of a functioning agroecosystem. Cover crop type and below-ground relationships dictate the rate and totality to which different plant litters decompose and thus how effectively their C molecules are redistributed.

7.2 Cover crops and AMF inoculum potential under-vine

Arbuscular mycorrhizal fungi are ubiquitous symbionts of more than 80% of earth’s terrestrial plants, with grapevines (*spp. V vinifera*) among their attractors. Though not essential for grapevine nutrition, AMF provide numerous vineyard ecosystem services, including nutrient availability for alternative vineyard plant communities (Smith & Read 2008). Our initial hypothesis suggested that increases in root biomass (cover crops) would inexorably lead to an increase in AMF colonisation and therefore AMF inoculation (rhizo-deposited spores and hyphae). Previous studies have shown mixed results – both suggestive that additional root systems increase AMF inoculum and that they have no significant effect versus an herbicide (bare earth) treatment (Baumgartner *et al.* 2010; Radić *et al.* 2012). We tested this hypothesis in a two-phase glasshouse trial. Initially, selecting leeks to grow as trap plants to test inoculum potential, we found no significant differences in the inoculum of soils sampled from beneath seven under-vine treatments. It was decided that leeks, although a suitable trap plant, were also highly mycotrophic and therefore we posited that any sources of inoculum that still resided in the herbicide-treated soil would instinctively colonise the leek roots. Therefore, a second experiment was conceived, this time testing AM inoculum potential on ryegrass and medic plants (one less mycotrophic species and one highly mycotrophic species). In both cases, we found no significant differences in colonisation, leading us to the conclusion that under these conditions we must be reject the hypothesis that AM inoculum potential is either increased under cover crops or decreased under herbicide management.

It was hypothesised that the herbicide treatment – with its lack of plant roots and potentially deleterious effect on AM inoculum – would significantly reduce the colonisation potential of trap plant roots. However, this was not the case. One explanation for these results suggests that the heterogeneity of the vineyard system allows for constant buffering of AM inoculum. That is, present *within* every treatment and *alongside* every treatment are grapevines *and* mid-row weeds. These constant sources of AM inoculum may play a vital role in buffering against any deleterious effects within the herbicide treatment. The vineyard is a heterogeneous system, prone to disturbance and therefore it can be difficult to isolate singular variables. Similarities are evident between these conclusions and those drawn within chapter six – cover crop decomposition and the *home-field advantage* (HFA). In that chapter, we concluded that the effects of HFA were likely dampened by the heterogeneity and frequency of disturbance (human and machine) within the vineyard system. These intrinsic vineyard characteristics (heterogeneity, disturbance and proximity of biotic variables) suggests that some buffering capacity may exist that dampens certain treatment effects.

One of the more salient points with regards to the results from chapter five pertains to the specificity of trap plant and therefore of cover crop in use. Although we found no significant differences in colonisation between under-vine soil treatments, we did find highly significant differences in colonisation of trap plant (i.e. medic and leek vs ryegrass). We observed far greater colonisation of medic and leek roots (highly mycotrophic) than ryegrass roots (less mycotrophic). Inoculum potential is just that – *potential* – and therefore in order to ensure AM colonisation and hyphal networks persist throughout the vineyard, bare earth should be planted. Specificity comes in to play when selecting the appropriate cover crop. To ensure the highest possible colonisation and AM proliferation, selecting a medic (or other mycotrophic plant) is recommended. This idea of cover crop *specificity* is paralleled once again in chapter six. Although we found no evidence to support HFA with regards to cover crop decomposition under-vine, we did find strong evidence to suggest that different cover crops decompose at different rates (as expected). This is of particular significance with regards to nutrient cycling and carbon turnover, especially regarding the mobility of transient N species during the decomposition process.

7.3 Cover crops and SOC: stocks and turnover under-vine

Chapters two and three explored the influence of under-vine management (specifically, cover crops) on soil organic carbon stocks, composition and turnover. Chapter three comprised a review paper on alternative vineyard floor management practices and SOC, whereas, chapter four presented primary research on SOC stocks, composition and turnover under-vine across two South Australian vineyards. Our hypotheses that soil under cover crops would have both greater SOC stocks and increased microbial activity was ultimately shown to be accurate. Several previous studies in Sicily, Spain, USA and Australia also found positive results for increased (or retained) OC both in the mid-row and under-vine (Ball *et al.* 2020; Fleishman *et al.* 2021; Ruiz-Colmenero *et al.* 2013; Steenwerth & Belina 2008). It was further hypothesised that labile OC – in the form of dissolved organic carbon (or DOC) – would also be increased under cover crop-managed soil and would correlate with microbial activity. However this was only the case at one vineyard site and at one depth layer. At Langhorne Creek (LC), between 0-10 cm, we found significantly higher DOC stocks. This disparity may be the result of site-specific management practice, with rates of irrigation at the LC vineyard far greater than at the Barossa Valley (BV) site. We postulate that increased rates of irrigation led to increased solubilised OC (DOC) in the shallower soil layer.

Specificity is a prominent theme in this discussion and one echoed throughout the previous chapters. Specificity is the antithesis of generality and I think it lends itself well to discussions on *terroir* and the multifarious variables that make vineyards unique. We found that treatments of fescue and clover (F+C) retained higher levels of SOC at LC; however, at BV there was no significant difference between treatments of F+C and medic and ryegrass (M+R). We speculated that this disparity was again likely due to increased rates of irrigation at LC, where fescue biomass was able to exploit excess water and produce more biomass. Specificity, in this case, might refer to cover crop selection versus regional climate (rainfall) and management practice (irrigation). In hot, dry, Mediterranean-type climates, vigorous grasses such as fescue may increase water competition, leading to a greater reliance on human inputs, such as irrigation. Where a vigorous grass planted alone may increase biomass at the expense of water and nitrogen competition, a grass planted in combination with a legume (i.e. medic + ryegrass) can be beneficial to SOC accumulation (Ball *et al.* 2020). Such findings are suggestive that the leguminous component of the combination is able to mobilise N, such that the grass component can increase biomass without depleting soil N – greater plant biomass leads to increased SOC (Ball *et al.* 2020).

The central theme of this thesis revolves around the notion of ecological intensification. Cover crops perform both primary and second ecosystem services. The review on SOC dynamics in vineyards (chapter three) discussed the role of cover crops as both *increasers* and *retainers* of SOC. The former suggests that increases in cover crop biomass directly correspond to increases in SOC stocks (Ball *et al.* 2020). The latter suggests that cover crops operate mechanistically, anchoring soil via root architecture and creating micro- and macro-aggregates, thus protecting sloping vineyards from erosion (Novara *et al.* 2011). We also discussed so called *co-benefits* of cover crop presence – the primary ecosystem services. In this, we highlighted the role of cover crops to improve infiltration, soil stability and structure – the primary ecosystem services (among more) (Ruiz-Colmenero *et al.* 2013). We know that cover crops increase and retain SOC and that SOC contributes towards water holding capacity, cation exchangeability, aggregate stability and reduced atmospheric CO_2 – the secondary ecosystem services (among more).

7.4 Cover crops and decomposition: HFA vs SMI under-vine

Understanding decomposition under-vine is beneficial to understanding SOC dynamics, namely, composition and turnover. Chapters two and three (SOC) discussed and measured SOC stocks, composition and turnover under-vine, while chapter six measured differences in decomposition (rate and totality) of cover crop litters in their *home* soil vs *away* soil. In each experiment heterotrophic respiration was measured, both as a function of litter quality (C:N ratio) and as a proxy for SOC composition. In both cases, litter quality was a key driver of decomposition dynamics, with medic litter (low C:N ratio) showing a steep initial curve, while easily-decomposable materials were readily metabolised. Materials with higher C:N ratios, such as wallaby grass, showed a slower initial spike; however, increased as more readily-decomposed compounds became accessible, eventually plateauing for longer as lower quality (less decomposable) residues were decomposed. Cover crop ecosystem services differ with varying qualities of plant litter and this can have consequences for nutrient cycling and C turnover dynamics in the vineyard.

Chapter six explored decomposition dynamics under-vine. Specifically, we were interested to understand whether cover crop residues would decompose at different rates – and the same extent – when buried in their own soil (*home*) versus the soil from a different under-vine treatment (*away*). The so-called *home-field advantage hypothesis* (or HFA) can be a useful theory to explain why some plant litters decompose faster and more completely in their own soil rather than a foreign soil (Austin *et al.* 2014). The theory has much to do with microbial endemism at the species plot level, and is thought to be most pronounced in homogenous ecosystems, where disturbance is less frequent (Gießelmann *et al.* 2011).

> *Interestingly, the idea to test HFA under-vine came to me as a result of efforts to mitigate the potential effects of HFA during experimentation into AMF inoculum potential (chapter five). The use of leeks was an attempt to select a suitable trap plant – one that was both mycotrophic and did not feature in the vineyard trial site. I then thought: "why not test the theory of HFA in and of itself?"*

A competing theory – though very much of the same coin – is the *substrate matrix interaction hypothesis* (or SMI). This theory can be regarded as an extension of HFA and posits that the quality of litter (C:N ratio, lignin content, etc.) is the driving force of decomposition (Veen *et al.* 2015). That is, microbial communities adapted to decomposing litters of a certain quality will also be adept at decomposing other litters of a similar type (Freschet *et al.* 2012). The theory is useful for explaining decomposition processes in more heterogonous and frequently disturbed systems, such as vineyards (Austin *et al.* 2014). Decomposition dynamics were measured both in the field (litter bag burial) and in the laboratory (litter incubation and heterotrophic respiration). In both cases, we found no significant differences in decomposition (rate and totality) between litters decomposed in their *home* soil vs their *away* soil. We concluded that potential effects of HFA were either dampened or non-existent in the vineyard and therefore suggest that SMI is a more likely driver of decomposition dynamics. Vineyards can be heterogeneous in plant type, with the addition of grapevine leaves, canes, grapes, cover crops and wild swards. Moreover, they are prone to frequent disturbance – both human and machine – which may inadvertently contribute to the movement of various decomposers throughout the vineyard, diminishing species-specific interactions.

This discussion has sought to revise the key findings of several interconnected hypotheses. Within the scope of this thesis, I have attempted to link these organs into one philosophical body. The core of which has revolved around the notion of ecological intensification – quantifying the various ecosystems services and co-benefits of three under-vine management practices (cover crops, straw mulch and herbicide). Within and between these vital organs is a network of fascia and interstitial fluid – linkages that provide connectivity and structure to the themes explored throughout. We can note the connection between SOC dynamics and decomposition under-vine. Soil organic carbon derives from plant residues entering the soil, with different residue types affecting residence time and the composition and turnover rate of SOC. We further note the theme of *specificity* as being crucial to sculpting a self-managing agroecosystem. The inoculum potential of AMF under-vine was explored and concluded that although AMF inoculum may be buffered by other sources, not all cover crops induce colonisation to the same degree. The specific use of mycotrophic cover crops (e.g. medic) show a significantly increased level of colonisation versus less mycotrophic plants (e.g. ryegrass).

7.5 Future directions

Mathematics must lie at the heart of scientific inquiry – after all, numbers don't lie. Physics is bound by the purest foundations of mathematics and, as such, it operates under finer, more deterministic margins. Ecology, on the other hand, is a science of biotic and abiotic interactions, where deterministic properties are immensely difficult to foresee and impossible to prove. Under these restrictions, ecology must operate under a stochastic framework – setting out a margin of probability and then testing to understand whether a hypothesis falls within said bounds. Within the vineyard trial sites we attempted to separate, distinguish and test specific variables of interest. It is always difficult to make bold assertions regarding the results we find and therefore we must be careful to state our findings within the specific spatiotemporal conditions of our trial sites. This long-winded preamble is to serve the purpose of stating that we are *quite sure* of certain outcomes and *somewhat less sure* of others. That is, there will always be future work, additions, amendments and revisions – this is scientific inquiry, after all, and it is meant to be a baton, passed from one inquirer to the next. To this end, I propose several key areas of future research that aim to both isolate variables more definitively and increase the core understandings set forth within this thesis.

1. <u>Further testing of AMF inoculum potential under-vine:</u>

   Initially, we tested AMF inoculum potential via the use of leeks as a trap plant. I had concerns that the intrinsic nature of leeks as highly mycotrophic plants had perhaps nullified any treatment-based results. Thus, we retested inoculum potential on two other plants (mycotrophic and non- mycotrophic). The non-significant results thereafter led us to suppose that AMF inoculum was being buffered by surrounding mid-row weeds or grapevines themselves. To test this more definitively, mid-row sources of inoculum could either be omitted from the study or included in the study as a treatment effect. In the former, this could be achieved either by maintaining bare-earth mid-rows for entirety of the trial. It could also be achieved by placing impermeable barriers between the mid-row and the under-vine to a depth beyond the weed root zone. In the latter, select sections of mid-row could be maintained as bare-earth, while others are allowed to maintain a wild sward. Ensuring it complies with the experimental design, this proposal could provide valuable information into the interaction between mid-row and under-vine cover crops and hyphal networks.

2. Completing the picture of SOC dynamics under-vine:

   In many ways the results pertaining to SOC stocks and turnover under-vine were the simplest and clearest of all. We were able to quite definitively show that cover crops increase SOC under-vine versus either a mulch or herbicide control. To better understand the role of leguminous cover crops and therefore of N, we propose that future studies should measure soil N throughout the year of experimentation in order to capture transient N species. However, the most pertinent aspect of future research into SOC dynamics is that regarding fractionation (physical and chemical) of SOC pools. We measured DOC pools across two sites but found that site-specific management practices may have masked treatment effects. Regardless of this, DOC represents only one SOC fraction and therefore in order to complete the picture of C dynamics under-vine, we propose further research. This could be undertaken with the use of select methods, including mid-infrared resonance spectroscopy (MIR) and even nuclear magnetic resonance spectroscopy (NMR). These methods can be used to discern the structural complexity of SOC molecules and thus broaden the picture of SOC composition under-vine.

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